The effect of long-lasting insecticidal nets on the transmission of malaria and lymphatic filariasis in Papua New Guinea, and opportunities for accelerating lymphatic filariasis elimination through novel treatment strategies

By

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Contents

List of figures and tables

Figures

Tables

Acknowledgments

This work is the culmination of a journey spanning ten years. It was never intended to comprise a PhD thesis, but through a relatively circuitous path, it has developed into something I am proud to present as such. First, the formalities: this work would not have been possible without financial support from the National Institutes of Health and the National Science Foundation in the United States, the Bill & Melinda Gates Foundation, and the Global Fund to Fight Aids, Tuberculosis, and Malaria.

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Submission Declaration

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. It has been composed by myself and has not been submitted in any previous application for any degree. The work presented (including data generated and data analysis) was carried out by the author except in the cases of the published works, which were collaborative. A statement of the author's contribution to these works can be found in Appendix 1.

Abbreviations

µm	micrometre
AE	adverse event
ALB	albendazole
ALT	alanine transaminase
AST	aspartate transaminase
ATP	annual transmission potential
DALY	disability adjusted life year
DDT	dichlorodiphenyltrichloroethane
DEC	diethylcarbamazine
DNA	deoxyribonucleic acid
EIR	entomological inoculation rate
ELISA	enzyme-linked immunosorbent assay
GEE	generalised estimating equations
GFATM	Global Fund to Fight Aids, Tuberculosis, and Malaria
GMEP	Global Malaria Elimination Program
GPELF	Global Program to Eliminate Lymphatic Filariasis
IDA	ivermectin, diethylcarbamazine, and albendazole
IRS	indoor residual spraying
ITN	insecticide-treated net
IVM	integrated vector management
IVM	ivermectin
km	kilometre
L3	third stage larva
LF	Lymphatic filariasis
LLIN	long-lasting insecticidal net
MDA	mass drug administration
MF	microfilaria
mL	millilitre
NTD	neglected tropical disease
PCR	polymerase chain reaction
PNG	Papua New Guinea
TAS	transmission assessment survey
WHO	World Health Organization

Summary

Currently, programmatic interventions to combat vector-borne diseases are largely underpinned by the concept that strategies proven effective in one context will work in all contexts. However, areas with high vector diversity present a challenge because the traits targeted by an intervention may not exist in most of the population. Papua New Guinea (PNG) is endemic for both malaria and lymphatic filariasis (LF) and has a diverse vector population. Typical vector control measures, such as long-lasting insecticidal nets (LLINs), have been very successful in reducing malaria burden in sub-Saharan Africa, partly because these mosquitoes bite humans at night inside houses. However, it may be that this intervention is inappropriate in areas like PNG, where a wide range of biting behaviour is the status quo, and that deploying complementary strategies will be necessary. The aim of this thesis is to evaluate the efficacy of existing and emerging interventions to prevent malaria and LF transmission and eliminate filariasis in PNG. This will be achieved through studies which 1) evaluate the impact of a nationwide LLIN distribution on vector density, behaviour, and species composition, 2) evaluate the impact of LLINs on malaria and LF transmission and disease prevalence and 3) evaluate the efficacy of complementary, integrated control measures to increase the likelihood of LF elimination. The data from observational and cross-sectional studies and a controlled clinical trial, demonstrate that LLINs can decrease transmission intensity of both malaria and LF in PNG. However, the sustainability of this control measure may be compromised by an epidemiologically significant shift in the behaviour of mosquitoes to bite at earlier hours of the night. Modifying LF elimination efforts, which typically include a mass administration of two drugs to the at-risk population, to incorporate a novel 3-drug regimen, is shown to potentially increase the likelihood of eliminating the disease.

Introduction

Anopheles mosquitoes

Mosquitoes in the genus *Anopheles* are arguably one of the most significant animals impacting human health. They are important vectors of human disease-causing pathogens, transmitting malarial protozoa, filarial nematodes, and less frequently, arthropod-borne viruses (arboviruses). Anopheline mosquitoes are distributed world-wide, but the most medically important vectors occur in tropical and sub-tropical regions[1], where higher temperatures allow relatively rapid development of parasitic infections. The most important vectors are closely associated with humans, having evolved search patterns targeting human-associated olfactory[2] and visual[3] cues. Understanding this behaviour has led to the development of the widespread vector control interventions long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), which exploit the tendency of these mosquitoes to seek out humans for a blood meal.

However, of the 41 different species of anophelines that transmit malaria[1], relatively few are completely anthropophilic. There is significant variation in host-seeking behaviour: some species tend to bite animals more than humans (zoophagy vs. anthropophagy), some bite outdoors more than indoors (exophagy vs. endophagy)[4], and some rest outside more than inside after bloodfeeding[5]. Even within a single species, behaviour can be quite variable. For example, host availability[6–8], the presence of vector control interventions[9,10], and seasonality[11] have all been shown to influence mosquito host-seeking behaviour. So while typical vector control tools are quite efficient at targeting a few important vector species, behavioural diversity and plasticity present major challenges to the sustainability of vector control[12].

Malaria transmission and epidemiology

Malaria is one of the most significant infections transmitted by anopheline mosquitoes. It has been estimated to cause 435,000 deaths in 2017[13] and is one of the leading causes of child mortality globally[14]. It is caused by the parasitic protozoans *Plasmodium falciparum, P. vivax, P. malariae, P. ovale,* and *P. knowlesi* which, upon entering a human host, infect liver and blood cells. The parasites undergo asexual reproduction within red blood cells, eventually bursting out of the cells and causing the high fever characteristic of malaria. Severe forms can quickly lead to organ failure, coma, and death. Some parasites develop into the sexual gametocyte stage within the red blood cells, which is the form capable of infecting a mosquito host.

If a mosquito ingests a bloodmeal with male and female gametocytes, they too will become infected with malaria. Once ingested, the gametocytes mate to form a diploid ookinete which embeds in the wall of the mosquito midgut to form an oocyst. Within the oocyst, meiotic division occurs, with subsequent mitosis to produce many sporozoites. These sporozoites migrate to the salivary glands of the mosquito, where they are injected into subsequent human hosts through the mosquito's saliva during the process of taking a bloodmeal. This process of asexual reproduction in the vector makes transmission of malaria efficient; the presence of a single oocyst in the midgut wall can produce a mosquito that injects many tens to over a hundred sporozoites during subsequent feeding attempts[15,16]. Therefore, a single bite from an infectious mosquito can lead to a malaria infection, with 5 infectious bites nearly guaranteeing infection[17]. Development of the parasite within the mosquito, the extrinsic incubation period, can take anywhere from 10 days to several weeks depending on the parasite species and ambient temperature.

Lymphatic filariasis transmission and epidemiology

Lymphatic filariasis (LF), another vector-borne disease, is a major cause of chronic disability though it does not contribute to significant mortality. It is caused by the filarial nematodes *Wuchereria bancrofti, Brugia malayi*, and *B. timori*[18]. The adult worms reside in the human lymphatic system, causing blockages that prevent the efficient movement of lymph. This causes lymphoedema of the extremities, elephantiasis, and hydroceles in men. LF infects approximately 64.6 million people and caused 1.36 million years lost to disability (YLDs) in 2017[19].

W. bancrofti is transmitted throughout most tropical and subtropical regions of the world and is co-endemic with malaria in many places[20]. It can be transmitted by several different genera of mosquitoes. *Anopheles* species are the primary vectors in rural Africa and the Southwest Pacific. *Culex* is the primary vector in India, Southeast Asia, Egypt, China, and some areas of East and Southern Africa. *Aedes* is important in the Pacific Islands[20].

Female adult worms residing in the lymph nodes release microfilaria (MF) into the bloodstream, which is the stage that is infectious to mosquitoes. When a mosquito takes a bloodmeal containing MF, they too may become infected[21]. Depending on the vector, MF are often periodic, only appearing in the blood at the time when vectors are biting. At other times, they sequester in the tissue of the lungs[22]. Because *Anopheles* mosquitoes are primarily nocturnal biters, *W.*

bancrofti in anopheline-transmitted settings is nocturnally periodic. Once ingested with a bloodmeal, MF escape the vector midgut by burrowing out of the midgut wall. They migrate to the thoracic muscles of the mosquito where they develop through two larval stages (figure 1)[23]. Once moulting to L3 larvae, they migrate to the head of the mosquito, where they burrow out of the mouthparts during blood feeding and actively penetrate the skin through the bite site[24]. Transmission of LF is relatively inefficient compared to malaria for several reasons. First, there is no reproduction within the vector; the maximum number of L3 that can develop is equal to the number of MF ingested[25]. In addition, there are several barriers to infection in anopheline mosquitoes, most notably the difficulty of bypassing the cibarial armature (a structure consisting of specialised "teeth" just anterior to the midgut) without being damaged[26,27]. Third, L3 are not directly injected with the saliva; rather, they are deposited on the skin and must actively find their way to the bite site. These factors, combined with many barriers within the human host including the necessity of adult worms to find a mate, has led to estimates that it takes many thousands of bites from infective mosquitoes to lead to an infection that produces MF[28]. These characteristics have led to confidence that LF can be eliminated once transmission falls below some threshold above zero[29]. This threshold is variable between sites, and depends, among other things, on vector species[30], existing human MF prevalence[30], and heterogeneity in mosquito exposure[31].

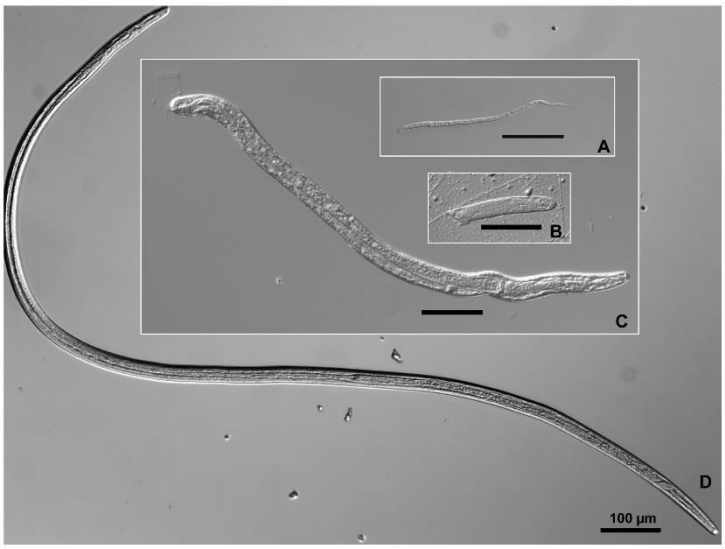

Figure 1 Relative sizes of the developmental stages of Brugia malayi *within mosquito hosts. Microfilaria (A) are ingested with a blood meal and escape the midgut. 1st (B) and 2nd (C) stage larvae exist in the thoracic muscles. 3rd (D) stage larvae are transmitted to humans[23].*

Control and elimination of LF and malaria

The cornerstones of control for both LF and malaria are transmission blocking interventions, although the adopted strategies for each disease are different. For malaria, the last 20 years has seen a vast increase in investment in vector control strategies, including insecticide-treated nets (ITNs), of which LLINs are

the most widely used, and IRS. LLINs are nets that are impregnated with a pyrethroid insecticide designed to last up to 3-5 years after passing certain tests of durability and quality designed by the World Health Organization (WHO)[32]. However, the effective life of a net depends the brand of net[33] and how it is used, including how many times it is washed[34,35], and whether it is exposed to sunlight[36]. When draped over a sleeping space, they protect the user from mosquito bites with a physical barrier as they sleep. In addition, at high enough coverage, ITNs provide community protection against malaria transmission by killing mosquitoes that come in contact with them[37]. The personal and community protection provided by nets consistently reduce malaria morbidity and mortality across multiple settings[38,39]. In addition, mathematical modelling predicts consistent reductions in parasite prevalence between 5-10% across multiple transmission settings in Africa at realistic coverage and adherence levels[40]. Epidemiological impact estimates suggest that ITNs are responsible for preventing 68% of the averted malaria cases between 2000-2015[41]. However, there is a clear trade-off between personal and community protection in cases where mosquitoes are not killed by nets, either through repellency, physiological resistance, or avoidance: those mosquitoes that avoid a user under a net are then free to seek a blood meal from an unprotected individual[42].

During IRS, interior walls of houses are sprayed with an insecticide formulation. The spray leaves a residue that can remain effective for up to ten months[43], although 2-6 months is more typical[44]. The effective duration depends on the insecticide formulation, target surface material and climate[45]. The effectiveness of IRS also relies on the behavioural trait of many mosquitoes to rest on the walls of houses after they take a bloodmeal. With a heavy abdomen full of the blood, mosquitoes cannot fly very well and require approximately 48 hours to digest the blood and produce eggs. While resting, the female is exposed to insecticide and is killed. The scale-up of vector control has had a dramatic impact on the burden of malaria, particularly in sub-Saharan Africa. Of all malaria control efforts, it is estimated that these two interventions have been responsible for 78% of the averted cases in the last fifteen years[41].

In addition to vector control, accurate diagnosis, treatment with artemisinin-based combination therapy, and preventative chemotherapy in vulnerable groups comprise the primary suite of interventions to prevent malaria[46]. Through these control tools, as well as the strengthening of surveillance systems and highly targeted control approaches, it is the global vision to eliminate malaria.

Towards this end, the WHO has set targets of reducing mortality and incidence of malaria by 90% in 2030 compared to 2015[46].

Rather that prevent transmission from mosquito to human, LF control efforts have focused on preventing transmission from human to mosquito. This is accomplished through a single annual mass drug administration (MDA) of the potent microfilaricides diethylcarbamazine (DEC) or ivermectin (IVM) with albendazole (ALB). By eliminating the MF reservoir, ongoing transmission is prevented. Because transmission is relatively inefficient, it is thought that a "worm breakpoint" exists: a threshold MF prevalence in the human population below which transmission is unstable and spontaneously moves to zero[29,30]. Unfortunately, the recommended MDA regimens are not very effective at killing adult worms, so annual MDA programmes must treat the entire at-risk population for at least 5 years, which is the estimated life span of the adult worms[47]. For this reason, current research efforts focus on identifying new drugs[48] or regimens[49] that are more effective against adult worms. Promising results have been obtained using an 8-week regimen of doxycycline[50], which targets the obligate bacterial endosymbiont *Wolbachia.* However, the prolonged treatment regime makes this a challenging strategy to implement at the community level. However, there is a potential "missed opportunity" for accelerating elimination with vector control[51–53] because there is a vector biting rate threshold that is analogous to the worm breakpoint[30]. Therefore, simultaneous deployment of MDA and vector control could be synergistic, since the effective implementation of one intervention lowers the threshold that the other intervention is required to attain to achieve elimination (figure 2).

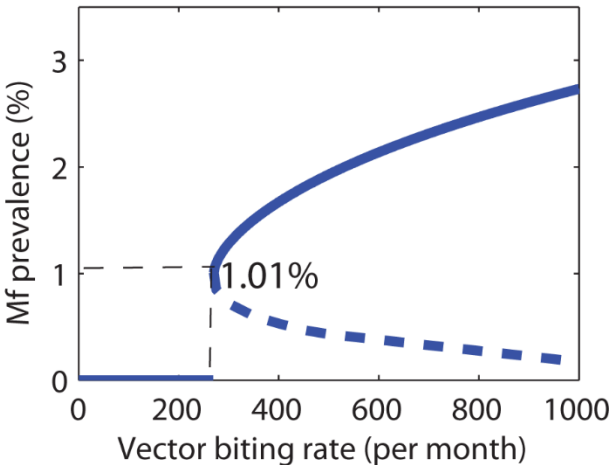

Figure 2 Depiction of the worm breakpoint values at various vector biting rates (dotted line). Solid lines represent the stable equilibrium MF prevalence at various biting rates. As biting rates decrease, worm breakpoints increase, indicating that vector control and MDA could work synergistically[30].

Similar to vector control efforts for malaria, MDA has been dramatically scaled up within the last 15 years. This is partly due to the commitment from drug manufacturing companies to donate the drugs required by MDA campaigns. This

made the goal of worldwide LF elimination a reasonable prospect, and the Global Program to Eliminate LF (GPELF) was launched in 2000 with an initial target of global elimination of the disease in 2020[54]. As of 2017, 7.1 billion doses of drug have been distributed[55], preventing 96.7 million cases of LF and decreasing the global prevalence from 3.55% to 1.47% between 2000-2012[56].

Control challenges

Although the strategies described above have greatly reduced the burden of malaria and LF, they are not without their challenges. For vector control, one of the largest challenges is the presence of insecticide resistance[57]. Resistance to multiple classes of insecticide has been selected for in many mosquito populations, and is now ubiquitous throughout sub-Saharan Africa[58]. Insecticide resistance has the potential to compromise the efficacy of insecticide-based control tool[59]. However, the evidence for this is scarce due to the difficulty of isolating the impact of resistance from other confounding factors[60].

Another challenge with these control tools is related to their targeting of specific mosquito behaviours, and their reliance on specific human behaviours. LLINs only target mosquitoes that bite humans, indoors, at night and IRS only targets those mosquitoes that rest indoors. While a significant proportion of mosquitoes have these characteristics, especially in sub-Saharan Africa, many do not. Exophilic and zoophilic behaviours are common amongst Asian malaria vectors[1]. Despite this, a recent meta-analysis did not find a significantly different impact of LLINs between African and Asian settings[39], although the Asian studies were restricted to India and China. In Papua New Guinea (PNG) and the Southwest Pacific, IRS was predicted to have a significant impact on malaria vector populations[61] due to their sufficient indoor resting habits. However, while IRS was able to completely eliminate some species, others were more resilient[9]. Pilot studies on bednets in PNG, with untreated bednets[62] as well as permethrin-impregnated nets[63], demonstrated that they had a significant impact on sporozoite positivity and biting rates. However, the magnitude decrease was relatively small (from 689 to 483 bites/person/night).

In addition, humans may decide not to use nets for a variety of reasons[64]. Even if they do use them, humans go to bed at various hours of the night and some are bound to be awake and out of their nets when mosquitoes are biting. Malaria transmission that still occurs despite the effective deployment and universal coverage of LLINs, or any vector control tool, is called residual malaria transmission[65]. The magnitude of residual malaria transmission in a location

depends on a number of factors, including the level of outdoor exposure, the propensity of vectors to seek non-human bloodmeals, and insecticide-induced avoidance of vector control measures, all of which can make mosquito populations more resilient[65].

In addition, there is increasing evidence that shifts in mosquito behaviour or composition may result in a post-intervention population that is less likely to come into contact with LLINs. At the closure of the Global Malaria Elimination Program (GMEP) of the 1950s and 1960s, which was largely driven by IRS with dichlorodiphenyltrichloroethane (DDT), one of the primary cited causes for the failure of the programme was behavioural resilience in *Anopheles* vector populations[66]. The primary difficulty in interpreting this data is the fact that many populations demonstrating behavioural shifts at the time were likely populations of morphologically indistinguishable species. Therefore, changes in behaviour within a population may have been due to taxonomic replacement. More recently, shifts to biting later in the morning have been seen in Benin[67], greater outdoor biting in Equatorial Guinea[68], and changes in species composition favouring those mosquitoes that do bite earlier in Tanzania[69]. These studies have benefited from modern molecular techniques allowing more accurate identification of species. However, all are still limited by the fact that they are observational studies and are unable to provide evidence of causation. In addition, there are relatively few studies documenting modern behavioural shifts despite the widespread use of LLINs. The underlying mechanism for these shifts is currently unknown. They may be caused by an opportunistic trait of some mosquitoes to find bloodmeals whenever or wherever they are available, with no particular fidelity to a particular biting time or location[63,70,71]. Alternatively, they may be durable evolutionary changes which are being selected for by vector control tools, as evidenced by a permanent change in biting behaviour in the Solomon Islands after the DDT spray campaign[9,72]. However, the genes that govern feeding behaviour in anophelines have yet to be identified[73]. Regardless, behavioural resistance has the potential to compromise the efficacy of control programmes[12], but there is currently little empirical evidence linking behavioural shifts to control failure.

Challenges associated with MDA largely relate to the difficulty in achieving high coverage for many successive years in a row. Coverage can be hindered by the inaccessibility of the communities[74], health system[75], political, or financial constraints[76], systematic non-access to certain individuals[77,78], and migration[79–82]. The inaccessibility of communities is particularly difficult to overcome, as it

not only results in fewer individuals receiving treatment, but a general distrust of the health system due to infrequent contact and hindered ability to communicate health promotion messaging[74]. In addition, although there are guidelines for when to stop MDA and begin surveillance for ongoing transmission (called transmission assessment surveys, or TAS[83]), the thresholds for transmission cessation are context-specific and often unknown[29,30,84]. Therefore, many countries have either had to continue giving MDA for many years beyond what was anticipated[85], or have found evidence of transmission despite meeting TAS-defined thresholds, resulting in the need to start over again[86].

The degree to which these challenges are experienced depends on the setting. Those areas that have more behaviourally "challenging" vectors stand to gain less from an LLIN intervention than those areas that have highly anthropophagic and endophagic vectors. Likewise, areas with strong political will backed up by appropriate financial resources and accessible populations will likely face significantly fewer challenges related to MDA compared to areas without these characteristics. This leads to the obvious question: why do homogeneous control efforts continue to be implemented across heterogeneous contexts, despite historical evidence indicating this may not be the best strategy?

Malaria and LF in Papua New Guinea

In Papua New Guinea (PNG), the challenges described above are at the forefront of the fight against vector-borne disease. Malaria and LF are both major problems in PNG. Malaria is perennially transmitted in the lowland and island areas, and the central highlands that run the length of the main island are prone to outbreaks[87]. A recent survey estimated a national mean parasite prevalence of 12.1%[88]. All four species of malaria parasite are actively transmitted in PNG and therefore coinfections of species and strains are common. However, only *P. falciparum* and *P. vivax* are the species associated with severe disease. Most of PNG's population is also at-risk of infection with LF, with only a few areas in the highland provinces where transmission is not stable. It is estimated that 18.5% of the population is MF positive, with over 40% positivity at some sites[89].

The major anopheline vectors of LF and malaria in PNG fall within a group of thirteen species called the *An. punctulatus* group[90]. Within this group are the species *An. punctulatus*, *An. koliensis*, *An. sp. nr punctulatus*, *An. clowi*, and *An. rennellensis*, and 8 members of the *An. farauti* complex, including *An. farauti s.s.*, *An. hinesorum*, *An. torresiensis*, *An. farauti 4*, *An. farauti 5*, *An. farauti 6*, *An. irenicus*, and *An. faruati* 8. Of these 13 group members, *An. punctulatus*,

An. farauti s.s., *An. hinesorum*, *An. farauti 4*, and *An. koliensis* are the major vectors due to their wide distribution and relative abundance compared to the others[91]. In addition, four other species have been incriminated as minor vectors of malaria, including *An. subpictus*, *An. karwari*, *An. longirostris*, and *An. bancroftii*. There may be even more than this, as *An. longirostris* has been shown to be a complex of nine species[92], and *An. bancroftii*, four species[93]. Each of these vectors exhibits different host-seeking behaviour and habitat preferences (Table 1), although all species tend to rest outside after blood-feeding[94]. Therefore, the species composition from site to site can vary substantially. Phenotypic resistance to DDT and pyrethroids has not been detected in any of the major malaria vectors across multiple areas of the country[95].

Table 1. Characteristics of the five major malaria vector species in Papua New Guinea.

Species	Distribution	Larval Habitat	Behaviour
An. farauti	Throughout PNG in lowland coastal areas[96]	Brackish water[96]	Bites throughout the night[97] with some tendency for early biting before 9pm[98]; feeds on different host species, depending on availability, with one study reporting a range of HBI between 9-83% between villages[94]; tendency for exophagy[98]
An. punctulatus	Throughout PNG in lowland inland areas and foothills[96]	Ephemeral pools, often associated with human disturbance[96]	Peak biting late night after 12am[97]; bites equally indoors and outdoors[99]
An. koliensis	Throughout PNG in lowland inland areas and foothills[96]	Ephemeral pools but also swamps and natural ground pools[96]	Peak biting late night after 12am[97]; will feed on animals but prefers humans where available[94]
An. hinesorum	Inland lowland	Natural ground	Assumed anthropophilic because of sporozoite

	regions[96]	pools[96]	rates[91]; peak biting in early night time hours before 9pm[97]
An. farauti 4	Inland lowland regions north of the central range[96]		Feeds on different host species, depending on availability[8]; bites throughout the night[97]

The GMEP was active in PNG beginning in 1958, primarily using IRS with dieldrin and in 1960, DDT. IRS with DDT continued to be scaled up until 1969, at which point over half of the at-risk population in PNG was covered, including all of the study sites in this thesis[100]. It was at this point that the goal of eradication was abandoned and a position of malaria control taken up[101]. Regular DDT spraying continued until the late 1970s, and in the early 1980s a programmatic evaluation determined that it was not effective in reducing malaria in lowland regions[102]. DDT was discontinued from 1984[101]. The DDT spray campaign in the Solomon Islands, a neighbouring island nation in the Southwest Pacific, witnessed behavioural resilience in the local population: mosquitoes exhibited a significant shift in biting times from the middle of the night to early evening hours. In addition, there was a significant shift to biting outdoors more than indoors[9]. Vector control efforts waned in the early 1980s, and continued at very low levels until 2004, when the PNG government secured a grant from the Global Fund to Fight Aids, Tuberculosis, and Malaria (GFATM) to complete the first nation-wide, free distribution of LLINs. These activities occurred between 2004 and 2009[103].

The Pacific Programme to Eliminate Lymphatic Filariasis (PacELF) was established in 1999[104], of which PNG is a member. However, MDA for LF has been sparse and restricted to various small-scale clinical trials to assess treatment efficacy[105–107], and a few successive annual rounds in the island provinces. Logistical and financial challenges have been numerous, and have prohibited large-scale, coordinated, nationwide MDA[89]. Small bednet trials indicated that untreated nets could reduce transmission of LF[108], but at the time of the LLIN mass distribution campaign, no comprehensive evaluation of the impact of a programmatic LLIN distribution on LF had been conducted in PNG or elsewhere.

Hypotheses, aims, and objectives

Between 2004 and 2009, PNG experienced its first large-scale vector control effort targeting malaria vectors since the DDT spraying of the 1960s. Since malaria vectors in PNG are behaviourally diverse and also transmit LF, this presented a valuable opportunity to test the hypotheses that 1) LLINs may not be as effective in areas like PNG, where a wide range of vector biting behaviour is the status quo, and 2) the impact of LLINs may extend beyond malaria to other vector-borne diseases like LF. Simultaneously, PNG has been struggling to sustain its MDA efforts, so even with vector control, 3) LF elimination may be more achievable with alternative drug regimens that could overcome challenges of typical MDA.

The overarching aim of this thesis is therefore to evaluate the efficacy of existing and emerging interventions to prevent vector-borne disease transmission and eliminate filariasis in PNG. This is addressed through three objectives:

1. to evaluate the impact of a nationwide LLIN distribution on vector density, behaviour, and species composition,
2. to evaluate the impact of LLINs on malaria and LF transmission and disease prevalence, and
3. to evaluate the efficacy of complementary, integrated control measures to increase the likelihood of LF elimination.

Summary of the studies

To achieve the aim of the thesis, a series of studies was completed from 2008-2013 in PNG (Table 2). Publication dates ranged from 2013-2017. A statement of the author's contribution to each publication, signed by all co-authors, can be found in Appendix 1. A full list of all the author's publications can be found in Appendix 2. The published works can be found in Appendix 3.

Table 2. Characteristics of the four publications submitted as part of this thesis.

#	Journal and date	Title	First author(s)	Co-authors
1	New England Journal of Medicine August 2013	Insecticidal Bed Nets and Filariasis Transmission in Papua New Guinea[109]	L. Reimer **E. Thomsen**	D. Tisch C. Henry-Halldin P. Zimmerman M. Baea H. Dagoro M. Susapu M. Hetzel M. Bockarie E. Michael P. Siba J. Kazura
2	Malaria Journal January 2016	Malaria transmission dynamics surrounding the first nationwide long-lasting insecticidal net distribution in Papua New Guinea[110]	L. Reimer	**E. Thomsen** G. Koimbu J. Keven P. Siba J. Kazura M. Hetzel P. Zimmerman
3	The Journal of Infectious Diseases March 2017	Mosquito behaviour change after distribution of bednets results in decreased protection against malaria exposure[111]	**E. Thomsen**	G. Koimbu J. Pulford S. Jamea-Maiasa Y. Ura J. Keven P. Siba I. Mueller M. Hetzel L. Reimer
4	Clinical Infectious Diseases February 2016	Efficacy, safety, and pharmacokinetics of coadministered diethylcarbamazine, albendazole, and ivermectin for treatment of Bancroftian filariasis[112]	**E. Thomsen**	N. Sanuku M. Baea S. Satofan E. Maki B. Lombore M. Schmidt P. Siba G. Weil J. Kazura L. Fleckentstein C. King

Each of the thesis objectives were informed by multiple studies, and some of the results from earlier studies informed future analyses and research. For example, results from studies 1 and 2 directly influenced the hypotheses and analysis methods presented in paper 3. Likewise, the results from paper 1 stimulated further research questions that informed the approach used in paper 4. The study characteristics, how they relate to the thesis objectives, and how they

relate to each other are shown in figure 3.

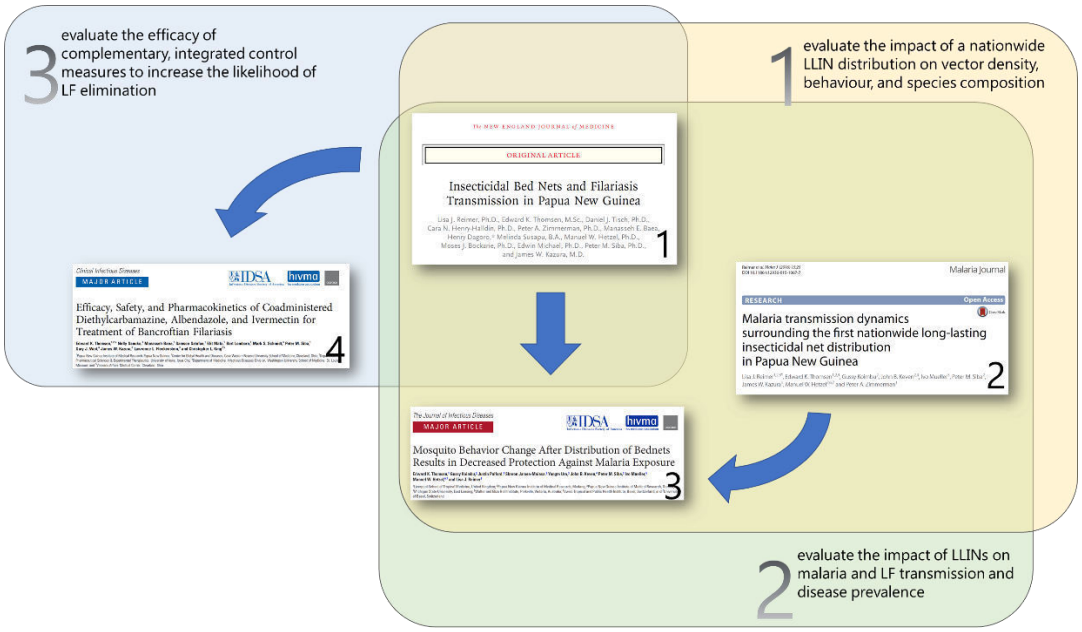

Figure 3 Venn diagram showing the three major objectives of the thesis (coloured boxes), and the papers that contributed to each objective. Arrows indicate where results from one paper influenced the approach taken on another.

A timeline for all studies is show in figure 4. Most of the sample collection occurred between 2008-2012, sample and laboratory analysis between 2009-2013, and data analysis and writing between 2011-2016.

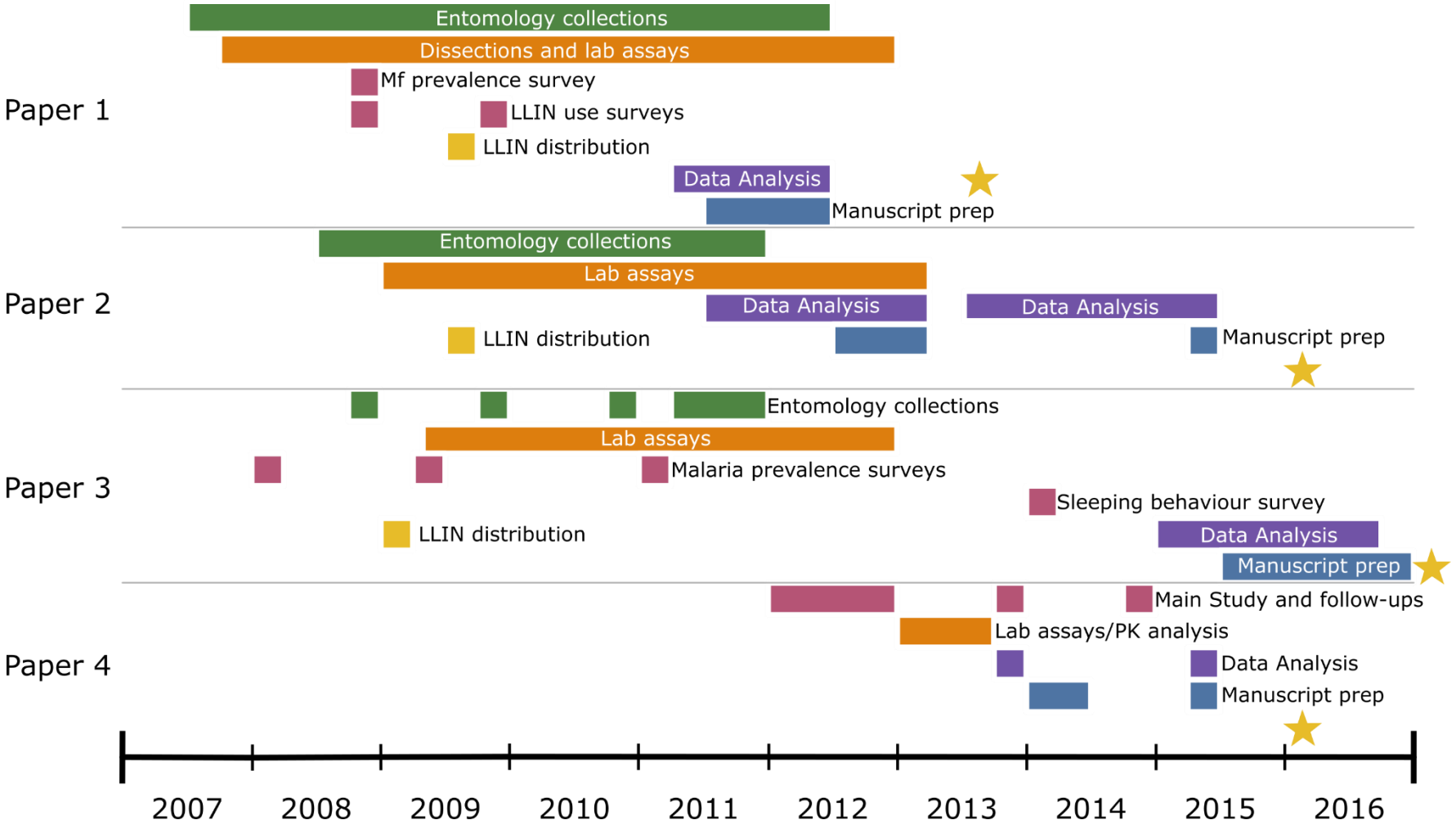

Figure 4 Timeline of various activities for each paper. Star indicates publication date.

The studies were completed in different parts of PNG. Paper 1[109] was conducted in five villages in the Dreikikir District of East Sepik Province, paper 2[110] was conducted at these five villages as well as six more along the north coast of Madang Province, paper 3[111] was conducted in three villages along the Ramu River Valley of Madang Province, and paper 4[112] was conducted at the Dreikikir Health Centre with individuals recruited from the village of Tau approximately 10 km south of the health centre (figure 5).

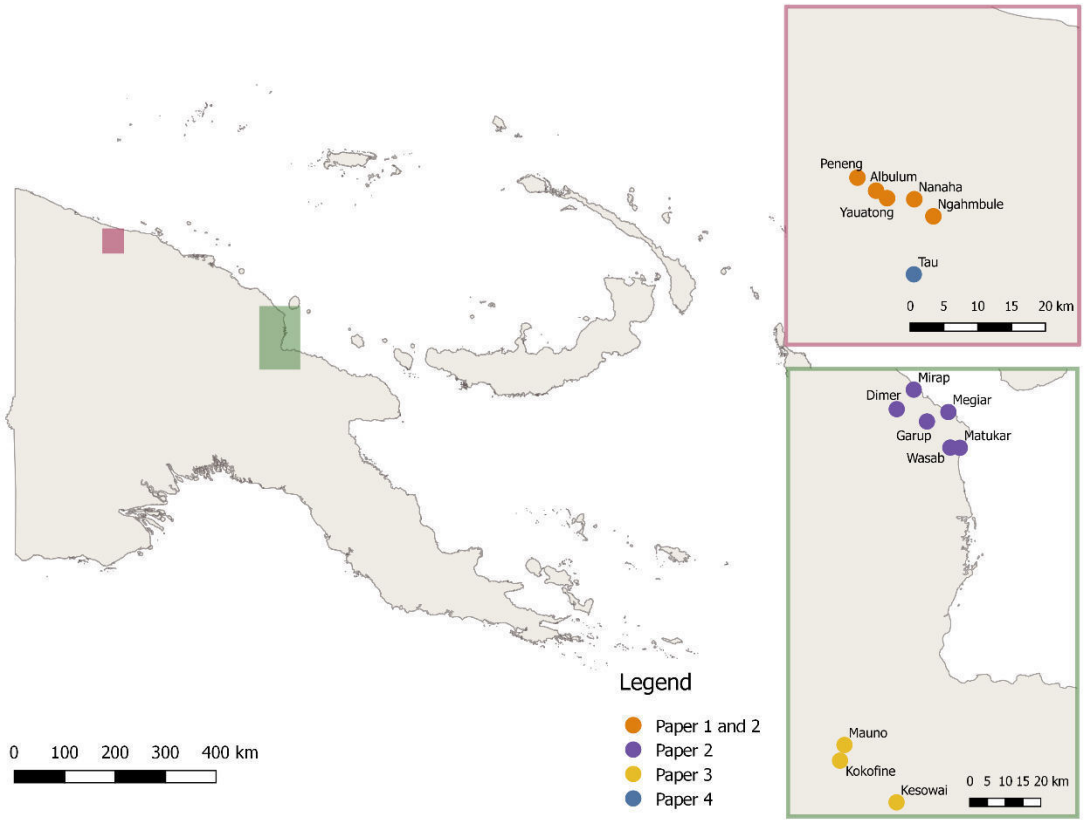

Figure 5 Map of Papua New Guinea with insets showing locations of study sites for the four papers.

Paper 1 - Insecticidal Bed Nets and Filariasis Transmission in Papua New Guinea

Rationale

The challenges of attaining high drug coverage in an LF MDA programme for multiple consecutive years have led to concern that a single global strategy of elimination may not be resilient, and that lack of vector control may hinder the progress of LF elimination[51,52,113,114]. There are many areas where *Anopheles spp*. transmit both LF and malaria parasites, providing the opportunity for malaria control efforts to benefit the GPELF. Currently, the most widely

implemented vector control intervention for malaria is LLINs. However, data are lacking on how LLINs will complement MDA to achieve LF elimination goals.

Methods

Entomological and epidemiological data on LF were collected from five villages in an area of PNG that had previously received MDA for five years ending in 1998[115], and which received LLINs as part of a mass distribution campaign in August of 2009. Mosquitoes were collected by human landing catch every month for 26 months before the LLIN distribution and 11-36 months after the LLIN distribution, depending on the village. Collected mosquitoes were stored according to the hour they were collected. All mosquitoes were morphologically identified, and a portion of the mosquitoes were confirmed to species and assessed for the presence of *W. bancrofti* DNA by PCR[116]. Another portion were dissected to look for *W. bancrofti* in various body tissues. A parasite prevalence survey was conducted approximately one year prior to the LLIN distribution among consenting adult volunteers and children >1 year old from the five study villages. Venous blood was drawn at night, 1mL was passed through a 5µm polycarbonate filter, the filter was stained, and the microfilariae were counted. LLIN use surveys were conducted in November 2008 and again in September through December 2009 by asking adults ≥18 years and children ≥1 year (or their parent/guardian) if they had slept under an LLIN the previous night. Daily human biting rates were estimated as the mean number of host-seeking anophelines collected by human landing catch in a 12-hour period. Daily transmission potentials were calculated by multiplying the daily biting rate per mosquito species in each village by the mean number of L3 larvae per mosquito. The mean daily value for each village was then multiplied by 365 to calculate the annual transmission potential (ATP), an estimate of how many infective L3 larvae are inoculated per person per year. To estimate the likelihood of transmission extinction, a previously developed population dynamic model of LF transmission[30] was fitted to age-stratified MF prevalence data collected from the five villages. Estimates of threshold biting rates were obtained from the model and compared to observed biting rates before and after LLIN distribution to determine the likelihood of transmission cessation.

Results

LLIN use shortly after the distribution was between 75-90% (n=911). 21,899 anophelines were collected over the 3-year period from 2007 to 2010; 20,345 during the 26 months before LLIN distribution and 1,554 during the 11 months after LLIN distribution. Overall, the proportion of anophelines positive for *W.*

bancrofti DNA decreased from 19.4% (n=761) before LLINs to 14.9% (n=248) after LLINs (Fisher's Exact, p=0.13). The decrease in mosquitoes infected with any stage larvae was more pronounced, with 1.8% *An. punctulatus* infected before, and 0.4% after LLIN distribution (n=8,859 mosquitoes dissected, Fisher's exact p=0.005). No mosquitoes containing infective L3 larvae were found in any village after LLIN distribution, so while ATP was similar in the two years prior to the LLIN distribution, it dropped to zero afterwards (figure 6). There was a significant shift in the biting time of mosquitoes to earlier hours of the evening, decoupling the previously close association between hourly biting rates and MF density in the blood (figure 7). The probability that transmission was interrupted was <1% in all five villages before LLINs. After LLINs, the probabilities increased to 4.9%, 7.7%, 90.5%, 95.8%, and 61.5%, and were higher in those villages with a lower MF prevalence prior to the LLIN distribution.

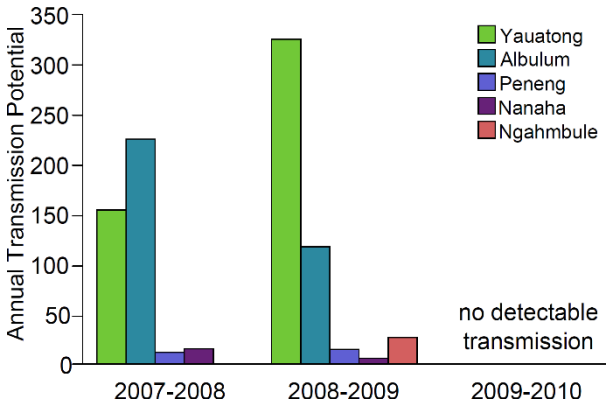

Figure 6 Annual transmission potential (L3 inoculated per person per year) before LLINs (2007-2008 and 2008-2009) and after LLINs (2009-2010). Modified from [109].

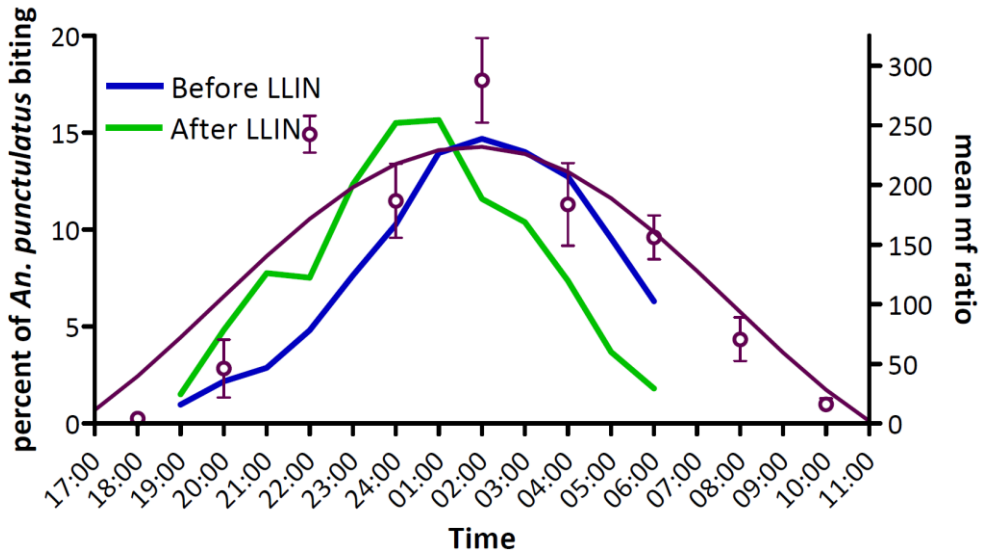

Figure 7 Changes in anopheline biting time following introduction of LLINs and nocturnal periodicity of microfilaremia in two Papua New Guinea study participants. Microfilaria (MF) ratios (as calculated in [117]*) are indicated by purple circles and a purple line. Anopheline biting times before LLINs were introduced are indicated by the blue line. Biting times after they were introduced are indicated by the green line*[109].

Summary interpretation and conclusion

This study documented LF transmission at monthly timepoints before and after an LLIN distribution. The decrease in mosquito abundance observed in the study is likely due to the LLIN distribution, although other factors, such as seasonal fluctuations or collector bias may have contributed as well. Collectors were local members of the community, and were therefore unblinded to the intervention and were in fact made aware of its intended impact. Regular monitoring of mosquito collectors was conducted throughout the study to ensure consistent methodology. Consistently low mosquito densities for up to 3 years after the LLIN distribution suggest that this was not caused by a natural event.

The proportion of mosquitoes positive for parasite DNA did not change significantly after the intervention, but the proportion positive by dissection decreased. This indicates that mosquitoes were still taking microfilaraemic bloodmeals, but were not sustaining parasite development. This may have been due to a decrease in the age structure of the mosquito population, a common impact of LLINs[118]. A decrease in life span would have prohibited the development of infective stage larvae within the mosquito population, which typically takes 13 days[27]. In addition, desynchronization of biting time and peak blood MF densities may have resulted in fewer MF being ingested, further decreasing the likelihood of a mosquito becoming infective.

The statistics used to analyse the data in this study did not incorporate methods to handle variation due to random effects. In addition, the data violated the statistical assumption that observations were independent. This may have obscured the true variation in the data likely caused by the presence of LLINs.

The population modelling suggests that the likelihood of transmission cessation was high in those villages with a low initial MF prevalence, indicating that LLINs may be an important stand-alone tool to reach elimination in these settings. Alternatively, they may act synergistically with MDA to help lower worm breakpoints.

Paper 2 - Malaria transmission dynamics surrounding the first nationwide long-lasting insecticidal net distribution in Papua New Guinea.

Rationale

PNG has a very diverse malaria vector population, with approximately five major vector species and an additional four important secondary vectors[90,91]. Each of

the five species exhibit different behavioural characteristics and habitat preferences[90,91,119]. In 2009, the PNG government distributed LLINs nationwide to prevent malaria transmission. However, the primary vectors bite both indoors and outdoors, humans and animals, and at various times of the night. With an intervention targeted primarily at late-night indoor biters, it was crucial to document the impact of LLINs on mosquito behaviour, abundance, and malaria transmission.

Methods

Mosquitoes were collected by human landing catch in 11 different villages in the Madang and Dreikikir Provinces of PNG. In Madang, three coastal and three inland villages were selected. The villages in Dreikikir were the same as those used in paper 1[109]. Collections were performed monthly in all villages for one year prior to the LLIN distribution and one year after the distribution, and continued on a bimonthly or quarterly basis in select villages for a third year. Mosquitoes were stored according to the hour, date, and household, identified morphologically, and species-confirmed by PCR[120]. To establish infection rates, mosquitoes were screened for *P. falciparum* and *P. vivax* circumsporozoite protein by enzyme-linked immunsorbent assay (ELISA)[121]. During collections, environmental conditions were also assessed on an hourly basis, including rainfall, wind, and cloud cover. In addition, the number of humans and animals present were counted. To identify the determinants of mosquito abundance and infection prevalence, generalised estimating equations (GEE) were used that included collector, house, village, date, and hour as random effects and presence of LLINs, rainfall, cloud cover, and wind as fixed effects (and mosquito species when the dependent variable was infection prevalence). Mean human biting rates were calculated per village and year, and post-hoc comparisons of biting rates within villages from year to year were performed with t-tests. Entomological inoculation rate (EIR) was calculated as the mean number of bites/person/year multiplied by the sporozoite prevalence. Median biting times were calculated per village per year and comparisons between years were made with Mann-Whitney U tests.

Results

Human biting rates significantly decreased in 8 of the 11 villages after the distribution of LLINs, and the EIR decreased in all but one village (figure 8).

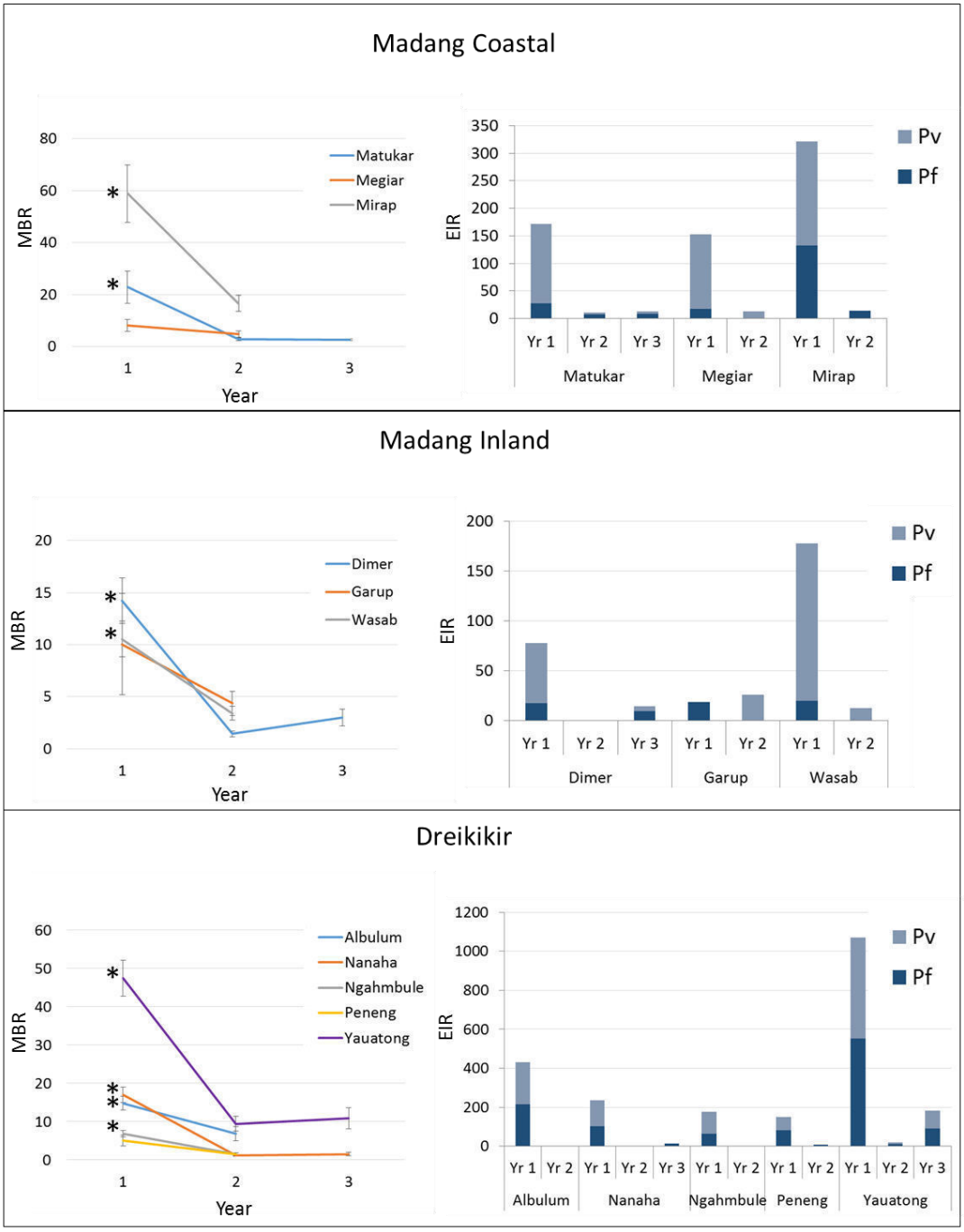

Figure 8 Anopheline biting rates (MBR, left side) and entomological inoculation rate (EIR, right side) in the three study regions. In all cases, year 1 is before the LLIN distribution and years 2 and 3 are after the distribution. Light blue bars on the EIR graph represent P. vivax and dark blue bars represent P. falciparum. Asterisks indicate villages where significant decreases in biting rates occured after LLIN distribution. Modified from [110].

In addition to LLINs, rainfall the previous month, cloud cover, and wind all had a significant impact on mosquito abundance. After LLINs, there was a significant shift to earlier biting times across multiple villages in both *An. punctulatus* and

An. farauti s.s. In addition, species composition significantly changed, with *An. farauti s.s.* increasing in proportion in coastal areas, and *An. punctulatus* increasing in proportion in inland areas. Sporozoite prevalence significantly decreased in the first year after LLIN distribution, but significantly increased in the second year after the intervention. Of the three major vector species found (*An. punctulatus*, *An. koliensis*, and *An. farauti s.s*.), when controlling for village and study year, all were equally likely to carry *P. vivax* sporozoites, but *An. punctulatus* was more likely to carry *P. falciparum* sporozoites.

Summary interpretation and conclusion

LLINs contributed to a significant reduction in mosquito abundance and malaria transmission across multiple mosquito species and locations, despite the presence of exophagic and early biting behaviours. Abundance remained low for over two years after the introduction of the intervention, with seasonal rainfall patterns staying consistent year to year. The impact of LLINs may have been due to their community effect in reducing exposure[122]. The intervention was also associated with changes in mosquito biting times, although the epidemiological significance of these changes requires further investigation.

EIRs were also reduced and stayed low for the duration of the study. However, this reduction was a result of the reduction in biting rates rather than sporozoite prevalence, because sporozoite prevalence rebounded between years 2 and 3 post-intervention. This observation may have been a result of a low sample size, which is frequently a challenge in post-intervention settings. However, it does demonstrate that there was considerable residual transmission, and that a resurgence in mosquito density would be accompanied by an increase in transmission intensity as well.

Although some statistical analyses (the GEE) included methods to effectively handle non-independence of observations, random effects, and non-normal response variables, other statistical analyses did not, such as the Mann-Whitney U test to look at shifts in biting times. A more comprehensive random effects model would have allowed further elucidation of sources of variation in the data and perhaps would have provided more power to detect differences.

Paper 3 - Mosquito behaviour change after distribution of bednets results in decreased protection against malaria exposure

Rationale

Historically, changes in mosquito behaviour in response to vector control interventions have presented challenges for malaria control[66,123,124]. Since 2000, bednets have dominated as the most widely used malaria vector control tool worldwide, and several studies have documented changes in biting behaviour associated with net distributions[68,69,72]. However, there is a relatively poor understanding of the epidemiological consequences of these changes.

Methods

Estimates of human exposure to mosquito bites and human malaria prevalence were investigated in 3 villages in the Ramu River Valley of Madang Province in PNG before and after an LLIN distribution in January 2009. Mosquitoes were collected in two of these villages by indoor and outdoor human landing catch, stored by hour of collection, and identified by PCR[120]. Lysates were analysed by ELISA[121] for the presence of *P. falciparum* or *P. vivax* sporozoites. Human movement inside and outside as well as sleeping patterns were assessed as part of a national household survey[125]. A malaria prevalence survey was conducted three times during the study: once before the LLIN distribution in 2008, and twice after the distribution in 2009 and 2011. These surveys were conducted in the same villages as the mosquito collections and one additional village in close proximity. Estimates of exposure to mosquito bites were calculated by multiplying the outdoor and indoor hourly biting rates by the proportion of the human population outside, inside, and in bed at each hour. These estimates were calculated for different age groups as well as for a theoretical net user and non-net user. Estimated values of exposure and protective efficacy for users and non-users were compared between age groups

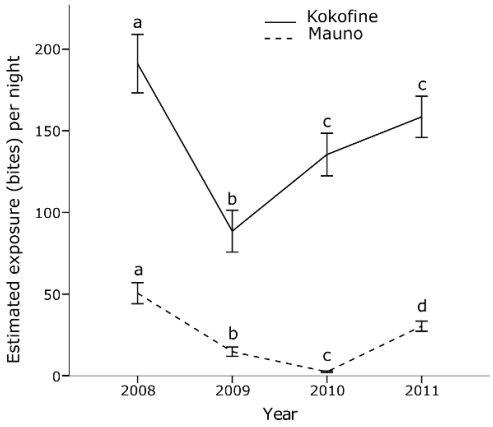

Figure 9 Total estimated exposure to bites for a non–net user before LLINs (2008) and after LLINs (2009-2011) in both study villages. Points sharing the same letter were not statistically different using a Kruskal-Wallis test with pair-wise comparisons. From [111].

and years using generalised linear mixed models, with year by age group as the fixed effect, and house and date of collection as random effects.

Results

A total of 41,757 anopheline mosquitoes were captured during the four-year period, with 99% of them identified as *An. farauti* 4. Biting rates initially decreased after the LLIN distribution but increased significantly in both villages within 2 years (Figure 9). The median time of biting in both villages was earlier after the LLIN distribution than before. Prevalence of malaria sporozoites was consistent across all four years. The true protective efficacy of nets was greater in younger age groups but decreased over the study period in all age groups due to the shift to earlier biting times and greater interaction with humans before bed time hours (figure 10). Only one village showed a consistent decrease in malaria prevalence from 2008-2011, with the other two villages either displaying no change or increasing (figure 11).

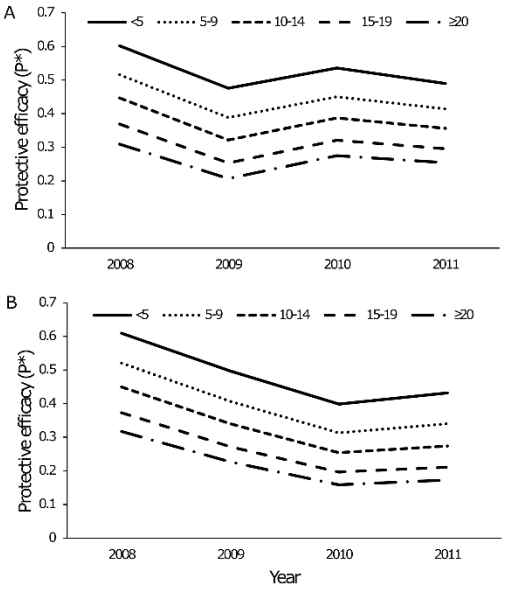

Figure 10 The protective efficacy of LLINs (the proportion of exposure prevented by using an LLIN) by age group in both Kokofine (A) and Mauno (B) villages before (2008) and after LLIN distribution (2009-2011). Modified from [111].

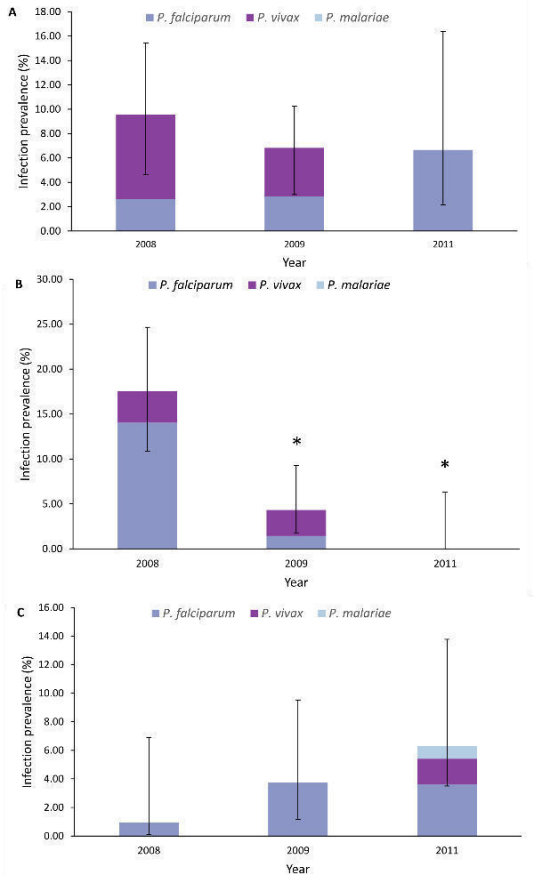

Figure 11 Human malaria prevalence in Kokofine (A), Mauno (B), and Kesowai (C) villages before (2008) and after (2009 and 2011) an LLIN distribution. Bars marked with an asterisk are statistically different from 2008 by a chi-square test. From [111].

Summary interpretation and conclusion

The data presented in this paper demonstrate a reduction in mosquito abundance immediately after the LLIN distribution, with a resurgence 2-3 years

later coupled with shifts to earlier biting. Estimations of mosquito-human contact taking into account the behaviour of both revealed that nets provided less personal protection after the distribution, because the shift in biting times resulted in more exposure during hours that people were awake and out of their nets. However, this study design had several limitations. First, paired indoor and outdoor mosquito collections were not conducted for the duration of the study. Previous studies have documented decreases in endophagy following the deployment of indoor interventions[69], which is a phenomenon that we were unable to capture. Second, human landing catches were not conducted before 6pm, so a significant amount of mosquito exposure may have been omitted from the analysis. If early and outdoor exposure was underestimated or if there was indeed a shift in endophagy, the analysis would have underestimated the magnitude of decrease seen in personal protection.

Other factors may have contributed to the resurgence in mosquito densities. First, environmental factors may have made for more favourable conditions in years 2 and 3. This study did not measure climatic conditions. However, rainfall data collected as part of paper 2 in Madang (~70km away from the study sites) indicated that there were no major seasonal patterns of rainfall. The study design, which included brief collection bouts separated by many months of no collections, may have highlighted week-to-week variation and obscured any long-term trends. Second, physiological resistance has been shown to decrease the operational impact of nets[126]. However, mosquito populations from this region of PNG have been shown to be susceptible to the insecticide used in LLINs[95]. Lastly, a decrease in LLIN use may have contributed to increases in mosquito densities, but based on two bednet surveys, the one included in this study and another independent survey in the same villages in 2012[8], LLIN use appears to have increased over this study period.

While LLINs did not have the intended impact on malaria prevalence, there was significant variation in malaria burden between villages and years. There may be many factors driving this variation. First, access to appropriate malaria diagnostics and treatment. Artemisinin combination therapy was only rolled out in this area at the end of 2011, and treatment failures with the previous combination of chloroquine plus sulfadoxine-pyrimethamine reached 18.5% in children infected with *P. falciparum* in PNG[127]. Therefore, the population may have been receiving inadequate treatment throughout the study period. Second, a higher baseline density of mosquitoes may make the population more resilient to control, which may be analogous to the well-known phenomenon of IRS

applications being more effective when deployed before the start of the malaria transmission season[128]. Finally, the study design did not allow measurement of seasonal fluctuations in malaria prevalence, so while the surveys were performed at the same time every year, the variation may have been typical. However, seasonal fluctuations in malaria incidence in this region have been shown to be remarkably stable[129].

Paper 4 - Efficacy, safety, and pharmacokinetics of co-administered diethylcarbamazine, albendazole, and ivermectin for treatment of Bancroftian filariasis

Rationale

The GPELF is grounded in the strategy of annual MDA for at least five years of ALB with either DEC or IVM[47], depending on co-endemicity with onchocerciasis, for which DEC is contraindicated. However, numerous challenges have resulted in some programmes failing to reach elimination targets with this strategy. Triple-drug therapy could have longer lasting microfilaricidal properties, but the efficacy, safety, and pharmacokinetic profile of IVM+DEC+ALB (IDA) has never been investigated.

Methods

A single-blinded, randomized controlled trial with two treatment arms (DEC 6 mg/kg + ALB 400 mg or DEC 6 mg/kg + ALB 400 mg + IVM 200 µg/kg) was conducted with individuals heavily infected with *W. bancrofti* in PNG. 24 individuals from Tau village in East Sepik Province met the inclusion criteria and were recruited to participate in the study. Participants were stratified by sex and randomly allocated to one of the two treatment arms. The night prior to treatment, a clinical assessment was performed, including physical examination, evaluation of lymphadenitis, lymphedema, and scrotal abnormalities in men, vital signs, urinalysis, blood hemoglobin levels, and subjective symptoms. The following morning, a blood sample was taken to evaluate baseline alanine transaminase (ALT), aspartate transaminase (AST), and creatinine levels, as well as drug levels. Blood draws were performed at 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, and 72 hours, and 7 days post dose. Thorough physical exams (as described above) were performed at hours 4, 8, 12, 24, 48 and day 7 post dose. The blood draws at 36 hours and 7 days were performed at night to assess MF levels. Subsequent night bleeds were performed 1 and 2 years after treatment. MF levels were assessed by passing 1mL of blood through a 5µm polycarbonate filter, washing and staining the filter, and counting the number of MF with a

microscope. Circulating adult worm antigen levels were measured using an ELISA assay[130].

Results

The percent reduction in MF was significantly greater in the IDA group compared to two-drug group at all follow ups (Figure 12).

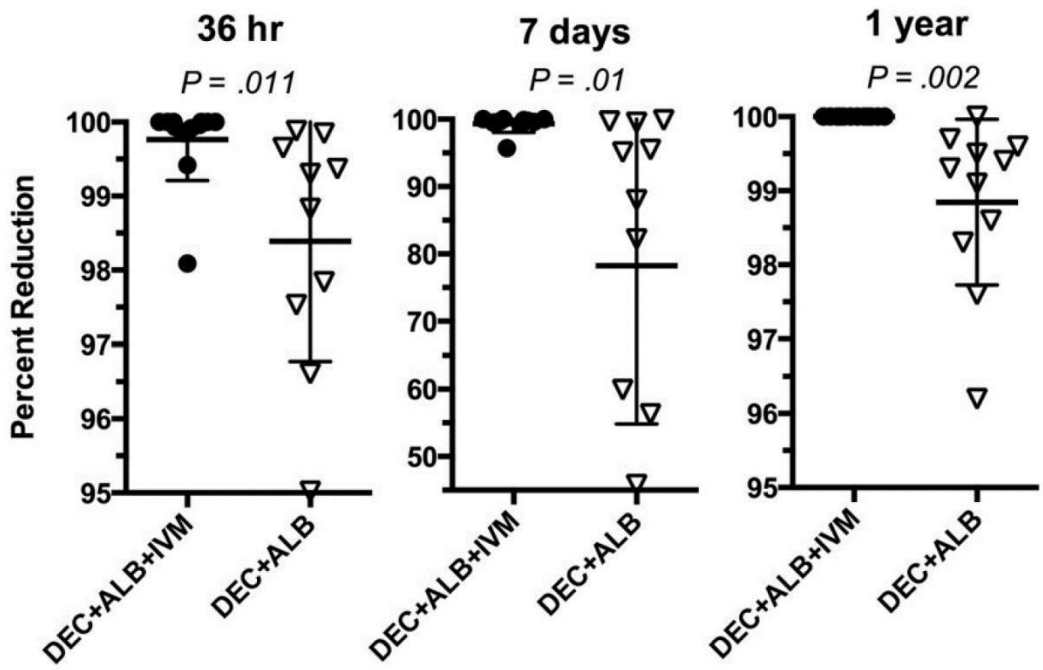

Figure 12 Percent reduction in microfilaria levels compared to pre-treatment at 36 hours, 7 days, and 1 year following treatment with either diethylcarbamazine, albendazole, and ivermectin (closed circles) or diethylcarbamazine and albendazole (open triangles)[112].

While 11 of the 12 participants in the two-drug group were MF positive 1-year post-treatment, all participants in the three-drug group were MF negative. The 6 individuals available for follow up after 2 years remained MF negative. Adult worm antigen levels decreased in both groups, but was more significant in the IDA group. Adverse events (AEs) were similar in both groups and consisted of fever, arthralgia, pruritis, and proteinuria/haematuria. All AEs (except fever, which was treated with paracetamol) were self-limiting and most dissipated within 7 days. Both groups had a similar proportion of participants experiencing any AE, but the median number of AEs was larger in the IDA group. Pharmacokinetic analysis indicated that there was no significant interaction between IVM and DEC or ALB.

Summary interpretation and conclusion

Participants in both groups showed reductions in MF densities. However, participants in the IDA group showed greater reductions and all of these participants who were available for follow up remained MF negative 2 years post

treatment. A greater reduction in adult worm antigen in the IDA group suggests that this treatment was more effective in killing adult worms. However, as both groups were antigen positive after treatment, the long-term reductions in MF densities seen in the IDA were likely due to embryostatic or embryocidal effects.

The AEs observed in both groups are consistent with previous studies that have documented the effects of mass killing of MF[131,132]. These include headache, fever, pruritis, arthralgia, and urine abnormalities. The IDA group had a greater number of AEs, which may have been due to greater efficacy against MF and adult worms. The pharmacokinetic parameter estimates of ALB (and its metabolites) and DEC were not different between treatment groups, indicating that IVM did not change the absorption or clearance of the other two drugs.

This study had a relatively small sample size, primarily powered to determine the presence of drug-drug interactions. Therefore, the preliminary efficacy data obtained in this trial must be verified with further studies.

Discussion

Impact of LLINs on mosquito abundance, behaviour, and composition

A large thrust of this thesis was the evaluation of how LLINs, which target endophagic and anthropophagic mosquitoes, work in a diverse vector environment. There has been a historic precedent that these types of "monotherapies" for vector control are inadequate[66,123,124,133]. The residual malaria transmission that remained after the DDT IRS campaigns of the 1950s and 1960s was one of the reasons cited for the ultimate failure of the GMEP[123]. Now that we are 15 years into the "age of the LLIN", it is important to quantify if and how LLINs are failing so that we are better informed to establish complimentary interventions.

In PNG, the first nationwide LLIN distribution campaign had dramatic successes. In paper 1[109] and paper 2[110], it was demonstrated that the abundance of vector species was significantly reduced after the distribution campaign. This impact was seen across several geographical regions of the country, and remained for up to three years after the intervention was in place. One result of this change in density was also a change in species composition, presumably because some vector species were more resilient to the intervention than others. This has also been documented in other areas, where the predominant vectors after LLINs are those that are more catholic in their biting behaviour[69,134]. These shifts in

composition may help to inform which species are likely to contribute the most to residual transmission of malaria and other vector-borne diseases in different regions of the country. Of the three major vectors in PNG, *An. koliensis* appears to be the most impacted by LLINs, followed by *An. punctulatus* and finally *An. farauti s.s.* This is understandable given the previously well-established early outdoor feeding behaviour of *An. farauti s.s*.[97].

A resurgence in mosquito abundance after an initial decline was only seen in the sites used in paper 3[111], where the dominant vector was *An. farauti 4* and there was an incredibly high baseline density of mosquitoes prior to the net distribution. One potential cause of this rebound may have been the shift in behaviour to biting at earlier hours of the evening. As a greater proportion of mosquitoes bite when humans are outdoors, these mosquitoes are then able to lay eggs and contribute to the next generation. Changes in biting times were also seen in papers 1[109] and 2[110] across multiple species, so understanding the impact of these changes over the longer term will be important to inform the continued use of LLINs. The relatively rapid rebound seen in paper 3[111] may have been a consequence of the already high mosquito population density seen here, and other areas may experience similar rebounds in the future. Other potential contributors to the rebound could have been a change in LLIN usage, a decrease in the effectiveness of the nets, or presence of physiological resistance in mosquitoes. However, these appear unlikely as net use remained high throughout the study period[8], LLINs remain effective for up to 5 years in typical use conditions in PNG[135], and there has been no detection of physiological resistance in mosquitoes of PNG[95,136].

In-depth analysis of the true exposure to mosquito bites, taking into account human behaviour and changing vector behaviour, revealed insights into the consequences of these behavioural shifts. The first insight relates to how personal protection can change with dynamic vector behaviour. Previously, a similar analysis indicated that despite behavioural shifts of *An. funestus* to feeding at early dawn, LLINs still provided high personal protection of >80%[137]. However, in paper 3[111], personal protection decreased significantly over the three-year period surrounding the net distribution, providing only ~30% protection at the end of the study. This is largely due to the temporal relationship between human outdoor activity and the scope of shift in biting times. If the shift occurs when people are still largely inside, as in Moiroux *et al*. 2014[137], then the protective efficacy of nets will change little. However, if the shift spans the time humans are rapidly moving from inside to outside, or vice

versa, as seen in paper 3[111], the behavioural shift will have a major impact on how much exposure can be prevented by using an LLIN.

The second insight relates to how behaviours of different subsets of the human population directly impact the personal protection of LLINs. Over the three-year study period in paper 3[111], true protective efficacy of LLINs decreased more in younger age groups than older age groups. Older individuals were largely outside until 10pm, and they therefore experienced a significant amount of outdoor exposure, both before and after the LLINs and associated changes in biting times. However, because younger individuals go to bed earlier, their exposure was more heavily influenced by the change in biting time to earlier hours.

Impact of LLINs on disease transmission and prevalence

Throughout most sites included in these studies, LLINs had the intended impact on disease transmission. LLINs contributed to reducing both LF and malaria transmission. In the case of LF, the impact on transmission was so great that infective L3 larvae were not detected at all during the post-intervention period[109]. This, in addition to the modelling results predicting a high probability of transmission cessation in villages with low/moderate endemicity, adds significant weight to the recommendation that LLINs should be used in combination with MDA to eliminate anopheline-transmitted filariasis[138]. Quantitative analyses and modelling play an important role in achieving the elimination goals of many neglected tropical diseases (NTDs)[139], and this paper demonstrated the potential to use models to predict the likelihood of elimination using entomological, rather than epidemiological, indicators. This may be a more cost-effective and ethical approach to verify absence of transmission in settings of continually decreasing prevalence, as an alternative to taking blood samples from young children to identify serological markers of ongoing transmission. However, one significant challenge of this strategy is the necessity to fit the model to age stratified MF prevalence rates, which are not available in most settings[84].

In the case of malaria, EIRs were significantly reduced in the year immediately after the net distribution, and in some cases for up to 2 years. It is unclear whether the magnitude of change in EIR is sufficient to substantially impact parasite prevalence, as the relationship between EIR and parasite prevalence is logarithmic[140] and large decreases in EIR at the higher range (from 300 or greater infective bites/person/night to ~30 infective bites/person/night) result in

minimal predicted change in prevalence. EIRs remained at ~10 infective bites/person/year after LLINs in most sites. An epidemiological survey conducted across the country evaluating the LLIN distribution documented a reduction in malaria prevalence in the general population from 15.7% to 4.8%[103]. However, even in those areas where transmission was reduced, sporozoite prevalence began to rebound, indicating a substantial amount of residual transmission still occurring. Perhaps more worrying was the lack of apparent effect of LLINs on malaria prevalence in paper 3[111]. This lack of impact was associated with a change in biting time which resulted in LLINs providing less personal protection. Although there is not a causal link between changes in mosquito behaviour and the lack of epidemiological impact of LLINs, it is a likely contributor. Another factor that was not measured in this study is net durability. It may be that due to typical use conditions, nets lose their functionality after 1-2 years, a result seen in African settings[34]. A similar study performed in PNG demonstrated that LLINs retain their insecticidal efficacy for 5 years[135], however while physical durability was measured in this study, it was not incorporated into an overall estimation of functional net survival times.

Complementary, integrated control measures to increase the likelihood of LF elimination

Despite 5 years of MDA in the PNG study villages from 1994 to 1998, paper 1[109] demonstrated that LF was not eliminated. This was evident by an ATP of up to 325 L3 inoculated per person per year prior to the LLIN distribution. This may have been a result of several factors, including migration of infected individuals into the communities[82], or the inability of MDA to surpass the MF positivity threshold (<1%) thought to lead to the absence of transmission[30]. The difficulties in achieving LF elimination despite sustaining high coverage for more than 5 years have been experienced elsewhere[85,141–143].

The studies presented in papers 1[109] and 4[112] of this thesis provide strong evidence that additional elimination tools exist that could effectively compliment current strategies to achieve global LF elimination goals. First, mathematical modelling in paper 1[109] predicted a high probability of LF transmission extinction in several villages due to LLINs alone. This finding has also been corroborated by a similar study in Nigeria[144]. LLINs could therefore work synergistically with MDA in areas with anopheline-transmitted LF, as long as they are able to keep biting rates low until adult worms die. These results call for greater integration between malaria and LF control programmes, which often work independently of one another.

Second, IDA eliminated MF from the blood rapidly and for up to two years post-treatment, which is a significant improvement on previous drug regimens. Although IDA may not be able to be implemented everywhere due to areas of sub-Saharan Africa co-endemic for onchocerciasis and loiasis, it could still be a valuable tool to increase the attainability of LF elimination goals. Although the study presented in this thesis was only powered to assess safety and drug interactions, the preliminary results are promising, and warrant conducting large-scale clinical trials to assess efficacy. If the long-term MF clearance is due to embryostatic or embryocidal effects on adult worms, it will be important to assess how long-lasting this effect is. Likewise, scrotal ultrasound[145] may help to determine if IDA has adult worm killing activity.

Significance and Impact

The studies included in this thesis have contributed significantly to furthering knowledge on control of the vector-borne diseases malaria and lymphatic filariasis. In addition, they have resulted in changes in policy recommendations at the global level that could see strategies for elimination shift entirely.

Paper 1[109] was the first to predict, using both empirical data and modelling, that LLINs have the ability to stop LF transmission entirely. This provided strong evidence to support integrated vector management (IVM) strategies between LF and malaria programmes, and has been cited in the WHO IVM handbook[146]. Paper 3[111] was the first study to associate shifts in mosquito biting times likely caused by LLINs with contemporaneous estimates of malaria burden. This is significant, as global policy makers continually express the need to use epidemiological, rather than entomological, outcomes as evidence on which to base decisions. This is likely to highlight behavioural resistance as a challenge worthy of global attention. Finally, paper 4[112] was the first to document the impact of IDA therapy on sustained suppression of microfilaremia in patients with LF. This work has stimulated significant additional funding to support large-scale clinical trials across 6 countries globally[147,148]. In addition, modelling studies using the data collected in this study predict that the IDA strategy could have a transformative impact on the global LF elimination effort[149,150]. In sub-Saharan Africa, using the current strategy of MDA and vector control, only 3 out of 36 countries will meet the target of eliminating LF by 2020, with 7 more before 2030, and the rest after that. Replacing standard MDA with IDA changes the picture entirely: 4 will achieve the 2020 target, and all countries will achieve elimination by 2025, with the median year being 2021[150]. As such, IDA is

currently recommended as the best regimen in areas not co-endemic for onchocerciasis[151], and there is strong momentum to perform additional safety trials in onchocerciasis endemic areas[152], given that the disease is on the decline and the likelihood of serious adverse events is waning.

Vector-borne disease control is at a crossroads. Many NTDs, like LF, are targeted for elimination. As prevalence declines, it will be important to tailor and continue to invest in control efforts to sustain the gains made already. For malaria, it is now well established that elimination will not be attainable without integrated vector control measures being applied using relevant evidence to inform decisions. These situations necessitate an abundance of high-quality research that is able to inform policy decisions. However, the ability to implement finely tailored control strategies, even with the relevant evidence, is hindered by numerous challenges, many of which are outside the purview of national programmes. A complex research-to-policy process slows down access to potentially cost-effective and equitable interventions[153]. Decisions are highly influenced by financing mechanisms, and global donor agencies therefore retain a large amount of power over which control strategies are implemented. Requiring that many different tailored strategies be approved by a global body is unrealistic, and therefore a certain amount of decision-making power must be transferred back to the countries that the interventions benefit. The capacity to make these decisions must therefore be bolstered, and analytical tools to assist in decision-making processes must be developed[154].

This body of work has contributed significant new insights that have already influenced the research and policy agendas for vector-borne disease control. However, further work is clearly needed to identify and evaluate potential vector control tools that could potentially overcome some of the challenges associated with behavioural resilience identified in this thesis. Tools such as ivermectin-treated animals[155] and transfluthrin emanators[156] show promise and could be beneficial in areas like PNG where pigs are commonly kept around human settlements[157] and where a large proportion of biting occurs outdoors and on non-human hosts[8]. A single treatment with IDA has recently been shown to be non-inferior to standard annual treatment with ALB and DEC in PNG for up to three years[158]. In addition, better diagnostics for *Loa loa*[159] and sophisticated mapping studies[160] may make it possible to better target IDA and other treatment strategies that are tailored to the local context. However, further work is needed to assess the operational feasibility of this approach.

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Appendix 1. Co-author statements

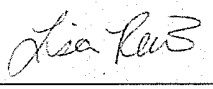
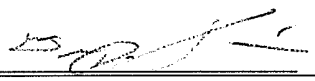
Edward Thomsen is submitting the following paper for consideration as part of a PhD by published work in the School of Life Sciences at the University of Warwick:

Reimer LJ*, Thomsen EK*, Tisch DJ, Henry-Halldin CN, Zimmerman PA, Baea ME, Dagoro H, Susapu M, Hetzel MW, Bockarie MJ, Michael E, Siba PM, Kazura JW. Insecticidal bed nets and filariasis transmission in Papua New Guinea. New England Journal of Medicine. 2013 Aug 22;369(8):745-53.

*equal contributors

Statement of contribution by Edward Thomsen:

EKT wrote the first draft of the manuscript, led the collection of epidemiological and entomological data, cleaned and analysed the data, revised the manuscript (with LJR and JWK), and monitored and performed laboratory analysis of samples (with LJR).

Name	Signature	Date
Lisa J. Reimer		23/05/2018
Daniel J. Tisch		10/7/2018
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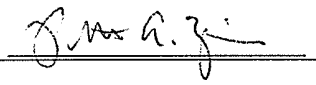
Peter A. Zimmerman		7/10/2018
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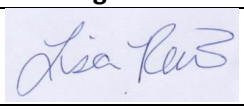
Lisa J. Reimer		23/05/2018
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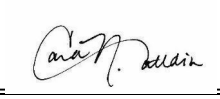
Cara N. Henry-Halldin		18/06/2018
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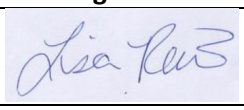
Lisa J. Reimer		23/05/2018
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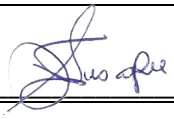
Melinda Susapu		25/06/2018
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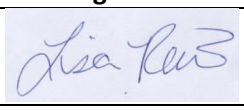
Lisa J. Reimer		23/05/2018
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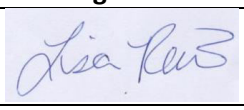
Lisa J. Reimer		23/05/2018
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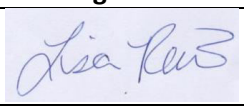
Lisa J. Reimer		23/05/2018
Daniel J. Tisch		
Cara N. Henry-Halldin		
Peter A. Zimmerman		
Mannaseh E. Baea		
Henry Dagoro	Deceased	
Melinda Susapu		
Moses J. Bockarie		
Edwin Michael		
Peter M. Siba		
James W. Kazura		24/05/2018
Manuel W. Hetzel		15.06.2018

I attempted to contact Manasseh Baea and Peter Siba via personal and professional email addresses. However, I was informed by the corresponding author on paper 4 that they have since retired and, as far as he is aware, are not able to be contacted.

Edward Thomsen is submitting the following paper for consideration as part of a PhD by published work in the School of Life Sciences at the University of Warwick:

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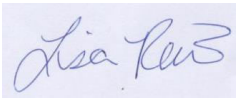
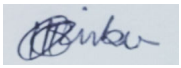
Lisa J. Reimer		_____23/05/2018_____
Gussy Koimbu		28/06/18
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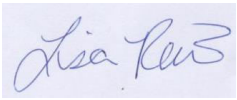
Lisa J. Reimer		_____23/05/2018_____
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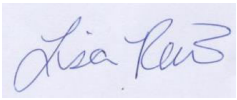
Lisa J. Reimer		_____23/05/2018_____
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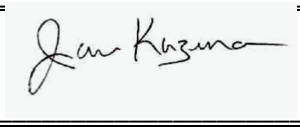
James W. Kazura		_____
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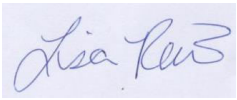
Lisa J. Reimer		_____23/05/2018_____
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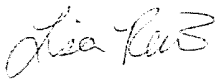
Lisa J. Reimer		23/05/2018
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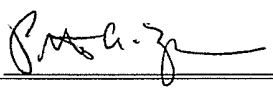
Peter A. Zimmerman		7/31/2018

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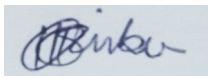
Gussy Koimbu		28/06/18
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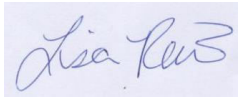
Lisa J. Reimer		23/05/2018

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Justin Pulford		18.6.2018
Sharon Jamea-Maiasa		
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Sharon Jamea-Maiasa		01.08.18
Yangta Ura		01.08.18
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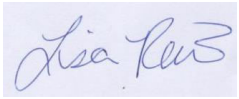
Lisa J. Reimer		_____23/05/2018_____

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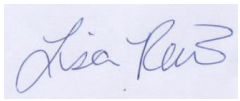
Lisa J. Reimer		23/05/2018

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Name	Signature	Date
Nelly Sanuku		26/06/2018
Mannaseh Baea		
Samson Satofan		02/06/2018
Elit Maki		26/06/2018
Bart Lombore		26/06/2018
Mark S. Schmidt		
Peter M. Siba		
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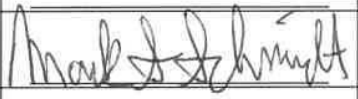
Mark S. Schmidt		June 28, 2018
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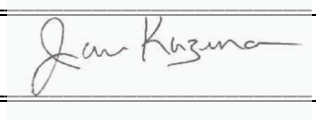
James W. Kazura		_____
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James W. Kazura		
Lawrence L. Fleckenstein	Lawrence L. Fleckenstein	June 21, 2018
Christopher L. King		June 12, 2018

Appendix 2. Edward Thomsen full bibliography

Thomsen EK, Hemingway C, South A, et al. ResistanceSim: development and acceptability study of a serious game to improve understanding of insecticide resistance management in vector control programmes. *Malar J*. 2018;17(1):422. doi:10.1186/s12936-018-2572-2

Mitsakakis, K., Hin, S., Müller, P., Wipf, N., Thomsen, E., Coleman, M., Zengerle, R., Vontas, J. and Mavridis, K., (2018). Converging human and malaria vector diagnostics with data management towards an integrated holistic One Health approach. International journal of environmental research and public health, 15(2):259.

Foster GM, Dunkley S, Deb RM, Thomsen E, Coleman M, Dhariwal AC, Das Gupta RK, Srikantiah S, Das P, Coleman M (2017). Adaptation of a malaria surveillance system for use in a visceral leishmaniasis elimination programme. International Health. 9(3):195-201.

Keven JB, Reimer L, Katusele M, Koimbu G, Vinit R, Vincent N, Thomsen E, Foran DR, Zimmerman PA, Walker ED (2017). Plasticity of host selection by malaria vectors of Papua New Guinea. Parasites & Vectors. Feb 21;10(1):95.

Thomsen EK, Koimbu G, Pulford J, Jamea-Maiasa S, Ura Y, Keven JB, Siba PM, Mueller I, Hetzel MW, Reimer LJ (2017). Mosquito behaviour change after distribution of bednets results in decreased protection against malaria exposure. Journal of Infectious Diseases. 215(5):790-797.

Foster GM, Coleman M, Thomsen E, Ranson H, Yangalbe-Kalnone E, Moundai T, Demba Kodindo I, Nakebang A, Mahamat A, Peka M, Kerah-Hinzoumbe C (2016). Spatial and Temporal Trends in Insecticide Resistance among Malaria Vectors in Chad Highlight the Importance of Continual Monitoring. PLoS One. 11(5):e0155746. doi:10.1371/journal.pone.0155746

Chanda, E*, Thomsen, EK*, Musapa, M, Kamuliwo, M, Brogdon, WG, Norris, DE, Masaninga, F, Wirtz, R, Muleba, M, Craig, A, Govere, JM, Ranson, H, Hemingway, J, Seyoum, A, Macdonald, MB, Coleman, M. (2016). An operational framework for insecticide resistance management planning for vector control. Emerging Infectious Diseases 22(5):773-79. doi: 10.3201/eid2205.150984

*these authors contributed equally

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Appendix 3. The published works

ORIGINAL ARTICLE

Insecticidal Bed Nets and Filariasis Transmission in Papua New Guinea

Lisa J. Reimer, Ph.D., Edward K. Thomsen, M.Sc., Daniel J. Tisch, Ph.D., Cara N. Henry-Halldin, Ph.D., Peter A. Zimmerman, Ph.D., Manasseh E. Baea, Henry Dagoro,* Melinda Susapu, B.A., Manuel W. Hetzel, Ph.D., Moses J. Bockarie, Ph.D., Edwin Michael, Ph.D., Peter M. Siba, Ph.D., and James W. Kazura, M.D.

ABSTRACT

BACKGROUND

Global efforts to eliminate lymphatic filariasis are based on the annual mass administration of antifilarial drugs to reduce the microfilaria reservoir available to the mosquito vector. Insecticide-treated bed nets are being widely used in areas in which filariasis and malaria are coendemic.

METHODS

We studied five villages in which five annual mass administrations of antifilarial drugs, which were completed in 1998, reduced the transmission of *Wuchereria bancrofti*, one of the nematodes that cause lymphatic filariasis. A total of 21,899 anopheles mosquitoes were collected for 26 months before and 11 to 36 months after bed nets treated with long-lasting insecticide were distributed in 2009. We evaluated the status of filarial infection and the presence of *W. bancrofti* DNA in anopheline mosquitoes before and after the introduction of insecticide-treated bed nets. We then used a model of population dynamics to estimate the probabilities of transmission cessation.

RESULTS

Village-specific rates of bites from anopheline mosquitoes ranged from 6.4 to 61.3 bites per person per day before the bed-net distribution and from 1.1 to 9.4 bites for 11 months after distribution ($P<0.001$). During the same period, the rate of detection of *W. bancrofti* in anopheline mosquitoes decreased from 1.8% to 0.4% ($P=0.005$), and the rate of detection of filarial DNA decreased from 19.4% to 14.9% ($P=0.13$). The annual transmission potential was 5 to 325 infective larvae inoculated per person per year before the bed-net distribution and 0 after the distribution. Among all five villages with a prevalence of microfilariae of 2 to 38%, the probability of transmission cessation increased from less than 1.0% before the bed-net distribution to a range of 4.9 to 95% in the 11 months after distribution.

CONCLUSIONS

Vector control with insecticide-treated bed nets is a valuable tool for *W. bancrofti* elimination in areas in which anopheline mosquitoes transmit the parasite. (Funded by the U.S. Public Health Service and the National Institutes of Health.)

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Lymphatic filariasis is a parasitic-worm infection caused by *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori* that affects approximately 120 million people in Africa, Asia, the Pacific, and the Americas.[1] Adult filarial worms live in the lymphatic system, causing lymphedema of the limbs, elephantiasis, and hydrocele. Fecund adult female worms release microfilariae, which ultimately enter the bloodstream, where they are ingested by anthropophilic mosquitoes of various genera. Microfilariae develop through several stages in mosquito vectors until they become infective larvae (L3), which continue transmission by establishing infection in humans through the bite site created during blood feeding. Safe, single-dose, inexpensive drug regimens have been developed that significantly reduce blood loads of microfilariae in humans for more than a year. For this reason, lymphatic filariasis has been targeted for global elimination by the year 2020 on the basis of annual mass administration of single-dose albendazole combined with either ivermectin or diethylcarbamazine for 5 or more years, the estimated reproductive life span of adult worms, which is anticipated to break the transmission of lymphatic filariasis from humans to mosquitoes.[2,3]

Although this effort has had successes, including the distribution of drugs to 570 million people in 48 countries,[4] it is faced with several challenges.[5] Annual treatment of at least 80% of eligible persons is key to elimination, but this level of population coverage has proved to be difficult to achieve in some areas because of health-system constraints[6] and human migration.[7] Financial and political limitations constrain the sustainability of control programs for lymphatic filariasis,[8] and there is the possibility of drug resistance developing in the parasite population.[9] Finally, elimination thresholds are site-specific and unknown in most areas.[10-12] Therefore, program managers in countries in which lymphatic filariasis is endemic may lack the evidence necessary to make informed decisions regarding whether to conclude, continue, or reinstitute mass drug-administration campaigns.

Heterogeneities in elimination thresholds for lymphatic filariasis are due largely to differences in vector–parasite relationships. In anopheline mosquitoes, the proportion of microfilariae that develop to become infective larvae decreases as the number that are ingested decreases, making this vector less efficient as the microfilaria reservoir diminishes, whereas the converse occurs in lymphatic filariasis transmitted by culicine mosquitoes.[13] In both systems, a decrease in the rate of mosquito bites will increase the worm breakpoint (i.e., the threshold below which the prevalence of microfilariae spontaneously moves to zero).[10] Therefore, the elimination of lymphatic filariasis becomes more attainable if vector control accompanies mass drug-administration campaigns. Elimination end points may also be affected by differences in local endemicity, infection aggregation, and the magnitude of acquired immunity.[10] For example, the worm breakpoint has been estimated to differ among neighboring villages in Papua New Guinea, where *Anopheles punctulatus* is the primary vector.[11] These difficulties suggest that a uniform global strategy for permanent cessation of transmission of lymphatic filariasis may not be resilient and that a lack of vector control may hinder progress toward this goal.[14]

The possibility of including vector control as part of programs to eliminate lymphatic filariasis has received increased attention.[15,16] In sub-Saharan Africa and Papua New Guinea, where anopheles species transmit both *W. bancrofti* and malaria, there is the opportunity to integrate the elimination of lymphatic filariasis with national malaria-control programs in which vector interventions are an essential component.[17] Observations made during malaria-eradication efforts in the Solomon Islands from 1974 through 1977 support the efficacy of vector control, since indoor residual spraying with insecticides decreased the prevalence of microfilariae from 22% to 0% without the use of antifilarial drugs.[18] Currently, the most widely implemented vector intervention used by malaria-control programs is universal coverage with insecticide-treated bed nets. However, only one study in Kenya has examined the effect of conventional permethrin-impregnated nets on *W. bancrofti* transmission by anophelines,[19] and there are no data that quantify how the use of bed nets treated with long-lasting insecticide will complement the mass administration of antifilarial drugs in reducing transmission of lymphatic filariasis and the probability of cessation of transmission.

We measured the transmission of lymphatic filariasis in five villages in the East Sepik Province of Papua New Guinea before and after a nationwide bed-net distribution effort in 2009. Communities in this area had received the last of five annual treatments with antifilarial drugs

more than 10 years earlier (1998) with no subsequent interventions until 2009. Since entomologic and human-infection data from this earlier time were available,[20] we were able to compare mass drug administration alone and the distribution of insecticide-treated bed nets alone with respect to the effect on the rate of transmission of lymphatic filariasis in the same region.

METHODS

STUDY AREA

Lymphatic filariasis is highly endemic in Papua New Guinea, where it is estimated that 4.4 million of the country's 6.3 million residents live in areas that qualify for disease elimination.[21] We selected five villages in the Ambunti-Dreikikir District of East Sepik Province for entomologic surveys and quantification of the prevalence of microfilariae (Fig. 1). Transmission of lymphatic filariasis in these villages has been well characterized.[20,22-24] We present our findings in the context of historical endemicity levels on the basis of annual transmission potentials (the number of infective larvae that were inoculated per person per year), which were measured before a trial of mass administration of antifilarial drugs conducted from 1993 through 1998.[22]

STUDY PARTICIPANTS

Mosquito collectors and village residents who participated in surveys of the prevalence of microfilariae and bed nets provided written informed consent after protocols were approved by the institutional review boards at University Hospitals Case Medical Center in Cleveland, the Institute of Medical Research, and the Medical Research Advisory Committee in Papua New Guinea.

PREVALENCE OF MICROFILARIAE

We measured the prevalence of microfilariae in 2008, before the distribution of insecticide-treated bed nets, by counting the number of microfilariae in a 1-ml sample of nocturnally collected venous blood after passing it through a 5-μm polycarbonate filter.

MOSQUITO COLLECTION AND ANALYSIS

Mosquitoes were collected monthly after landing on human adult collectors from July 2007 through July 2010. The collectors sat outdoors within several meters of the household entrance from 6 p.m. to 6 a.m. with their lower legs and feet exposed. Collectors worked in teams, with one member collecting from 6 p.m. to midnight and the other from midnight to 6 a.m. Mosquitoes landing in search of a blood meal were captured with an

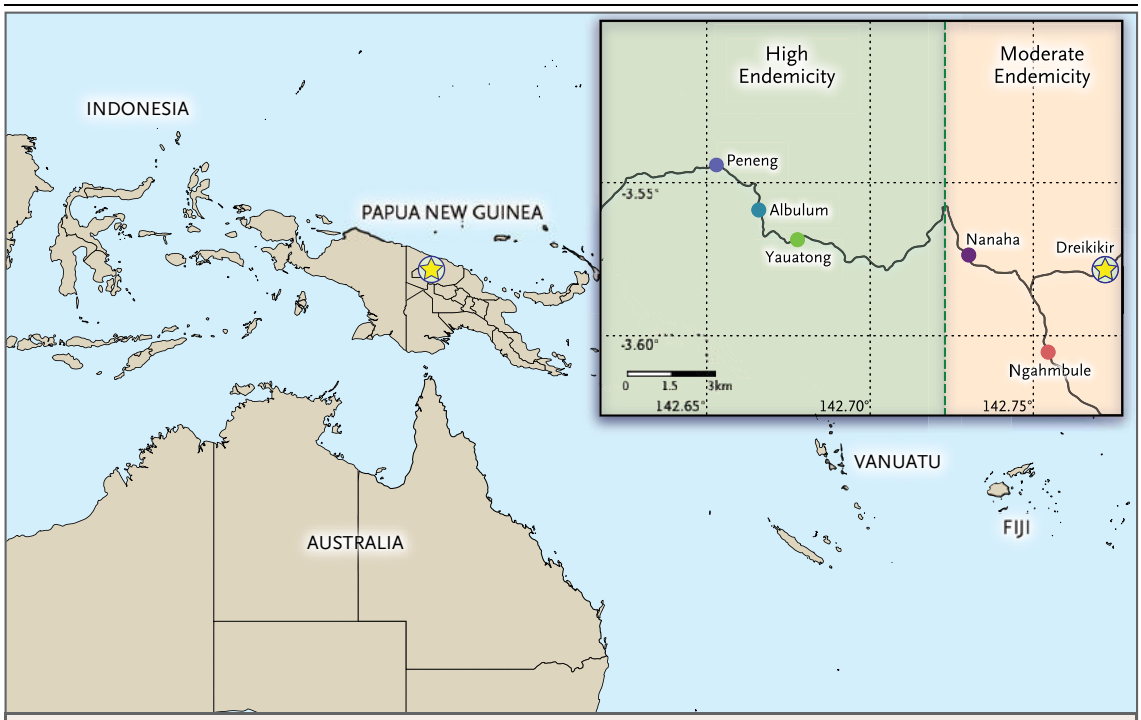

Figure 1. Location of Study Villages in East Sepik Province, Papua New Guinea.

aspirator and stored according to the hour they were collected. Each village was divided into four hamlets, with monthly collections in each hamlet. The total effort each month varied from 40 to 48 collection nights. Mosquitoes were morphologically identified as *A. punctulatus*, *A. koliensis*, or *A. farauti sensu lato*, according to criteria established previously,[25] and were stored according to species, location, and hour collected. Half the mosquitoes were stored in 70% ethanol for later dissection and the other half on silica gel for DNA diagnostic evaluation, as described below. A total of 10,578 mosquitoes were stained individually with Mayer's hemalum,[26] separated into body sections on a glass slide, and dissected with forceps and needles under a microscope. Dissected specimens were examined for *W. bancrofti* in the infective larval stage and other developing larval stages (L1 and L2) with the use of standard criteria.[27]

Genomic DNA was extracted from unfed dried mosquitoes singly or in pools of two (Qiagen). Identification of the species of 2867 mosquitoes was confirmed with the use of polymerase-chain-reaction (PCR) amplification of the internal transcribed spacer 2 region of mosquito ribosomal DNA and either digested with *MSP1* enzyme[28] or used in a ligase detection reaction–fluorescent microsphere assay.[29] A total of 1009 samples were also screened for *W. bancrofti* DNA by PCR amplification of the long DNA repeat region to be used as a xenomonitoring tool.[30]

At the close of the study, one village with a moderate transmission level (Nanaha) and one with a high transmission level (Yauatong) were selected for long-term assessment of rates of mosquito biting. Mosquito collections were conducted quarterly in the second and third year after the bed-net distribution.

INSECTICIDE-TREATED BED NETS

PermaNet 2.0, an insecticide-treated bed net impregnated with 55 mg of deltamethrin per square meter (Vestergaard Frandsen), was distributed to study communities by the East Sepik Province Division of Health in August 2009. At that time, the national target for bed-net coverage was 80% of household ownership and 80% use for children under the age of 5 years and for pregnant women. Surveys regarding bed-net use were conducted in November 2008 and again in September through December 2009 by asking adults (≥18 years of age) and parents or guardians of children if they had slept under a bed net the previous night.

STATISTICAL ANALYSIS

We estimated the daily mosquito-biting rates on the basis of the mean number of host-seeking anopheline mosquitoes that were collected in a 12-hour period. We calculated annual transmission potentials by multiplying the mean daily biting rate in the community by 365, which was then multiplied by the proportion of bites that were infective and by the mean number of L3 larvae per infective bite. We used the Mann–Whitney U test to compare biting rates before and after bed-net distribution and Fisher's exact test to compare rates of mosquito infection and infectivity before and after bed-net distribution. All statistical analyses were performed with the use of PASW Statistics, version 17.0.3 (IBM).

We used a numerical-modeling and Bayesian analysis method that was based on the mosquito-biting rate and the prevalence of microfilariae, stratified according to the age of residents in the 2008 survey, to estimate the likelihood of transmission cessation before and after bed-net distribution.[10,11] (Details are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.)

RESULTS

PREVALENCE OF HUMAN INFECTION

Descriptive characteristics of the study population[20] are summarized in Table 1. In 2008, the prevalence of microfilariae in the three study villages in the high-transmission zone ranged from 23.7% to 38.6%. These values were significantly higher than in 1998, when the values ranged from 3.7 to 10.8% ($P<0.001$ by Fisher's exact test), 1 year after the fourth annual mass administration of antifilarial drugs and immediately before the fifth and final mass treatment. In contrast, the prevalence of microfilariae in the two villages in the moderate-transmission zone, Nanaha and Ngahmbule, remained low and did not change significantly during the 10-year period, with prevalences of 3.4% or less in both villages in 2008 ($P=0.78$ and $P=0.39$, respectively, for comparisons with 1998 values). Notably, the prevalence of microfilariae in 2008 did not increase in any of the villages to the level in 1994, before mass drug administration (Table 1).

Table 1. Status of Lymphatic Filariasis and Use of Insecticide-Treated Bed Nets in the Study Villages.*

Level of Transmission	Village Name	Microfilariae Prevalence			Population of Village	Bed-Net Use before Distribution in 2009	Bed Nets Distributed	Bed-Net Use after Distribution
		1994	1998	2008				
		%			*no.*	%	*no.*	%
High	Yauatong	79.5	10.8	38.6	408	12.4	190	84.3
High	Albulum	78.3	7.4	38.4	526	NA	234	81.7
High	Peneng	61.5	3.7	23.7	233	3.8	142	75.0
Moderate	Nanaha	48.3	2.4	2.0	507	NA	222	84.1
Moderate	Ngahmbule	36.2	1.7	3.4	256	NA	109	90.6

* Transmission levels are based on annual transmission potentials determined in 1993 and 1994. Five annual mass antifilarial treatments consisting of diethylcarbamazine alone or diethylcarbamazine plus ivermectin were administered from 1994 through 1998. (This regimen differed from the standard treatment in the global program to eliminate lymphatic filariasis.) Insecticide-treated bed nets were distributed in August 2009, with a target coverage of at least 80% of households per village. NA denotes not available.

USE OF INSECTICIDE-TREATED BED NETS

Immediately before bed-net distribution, 3.8% and 12.4% of households of the two study villages surveyed used bed nets of any type. Four to 5 months after distribution, self-reported household use of bed nets in the five study villages ranged from 75.0 to 90.6% (Table 1).

MOSQUITO VECTORS

A total of 20,345 anopheline mosquitoes were collected in the 26 months before bed-net distribution and 1554 in the 11 months after distribution. The subgroups of mosquitoes in which the species was confirmed included 78% of *A. punctulatus* and 21% of *A. koliensis;* the remaining 1% was a mix of *A. hinesorum*, *A. farauti 4*, and *A. farauti sensu stricto*. Among *A. koliensis* mosquitoes, 94% were caught in Nanaha and Ngahmbule; molecular confirmation of the morphologic identification of *A. punctulatus* resulted in 95% concordance. Only mosquitoes that were identified as *A. punctulatus* on morphologic analysis harbored *W. bancrofti* infective larvae. Subsequent data are therefore based on morphologically identified *A. punctulatus*.

The proportion of *A. punctulatus* mosquitoes that were infected with any stage of larvae decreased from 1.8% to 0.4% after bed-net distribution (P=0.005 by Fisher's exact test) (Fig. 2). None of the mosquitoes that were collected in Peneng, Nanaha, or Ngahmbule after bed-net distribution contained larvae of any stage. Notably, no mosquitoes harboring infective larvae were identified in any of the villages after bed-net distribution (P=0.07 by Fisher's exact test). Therefore, although annual transmission potentials were similar during each of the 2 years preceding bed-net distribution, that number dropped to zero for the year after bed-net distribution (Fig. 3). The proportion of anopheline mosquitoes that tested positive for *W. bancrofti* DNA, an indicator of the reservoir of microfilariae, was 19.4% (761 mosquitoes) before bed-net distribution and 14.9% (248 mosquitoes) after bed-net distribution (P=0.13 by Fisher's exact test).

MOSQUITO-BITING RATES

The daily biting rates for anopheline mosquitoes decreased significantly after bed-net distribution, with a mean (±SE) of 61.3±4.9 bites per person per day before bed-net distribution versus 9.4±1.9 after bed-net distribution in Yauatong, 22.6±2.5 versus 7.3±2.0 in Albulum, 6.4±0.8 versus 1.5±0.4 in Peneng, 21.5±1.3 versus 1.1±0.2 in Nanaha, and 8.9±0.8 versus 1.5±0.3 in Ngahmbule (P<0.001 for all comparisons by the Mann–Whitney U test). The rates remained consistently low for an additional 2 years in Yauatong and Nanaha (Fig. 4).

The probability of the transmission cessation was less than 1.0% in all five villages before bed-net distribution. After bed-net distribution, the probabilities increased to 4.9%, 7.7%, 90.5%, 95.8%, and 61.5% in Yauatong, Albulum, Peneng, Nanaha, and Ngahmbule, respectively. Further reductions in biting rates by years 2 and 3 after bed-net distribution increased the probabilities to 36.8% and more than 99% in Yauatong and Nanaha, respectively. These high probabilities provide support for the empirical finding that annual transmission potentials were reduced to zero after bed-net distribution (Fig. 2).

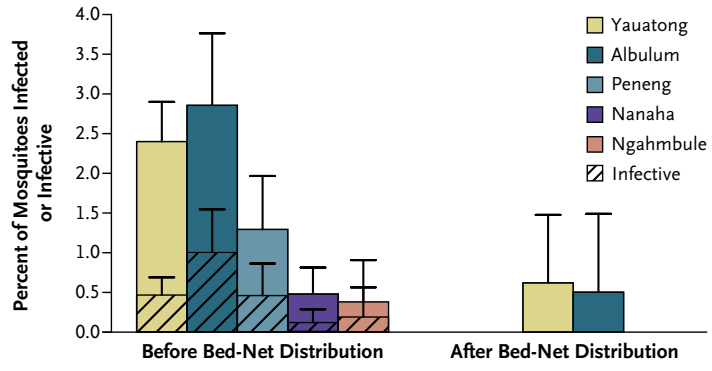

Figure 2. Proportion of *Anopheles punctulatus* Mosquitoes Carrying Nematodes Causing Lymphatic Filariasis, before and after the Distribution of Insecticide-Treated Bed Nets in Five Villages in Papua New Guinea.

Shown are the percentage of mosquitoes that were found to be infected (solid colors) and the percentage that were found to be infective (hatched areas) on dissection before bed-net distribution (8181 mosquitoes) and after bed-net distribution (678 mosquitoes). The T bars indicate 95% confidence intervals.

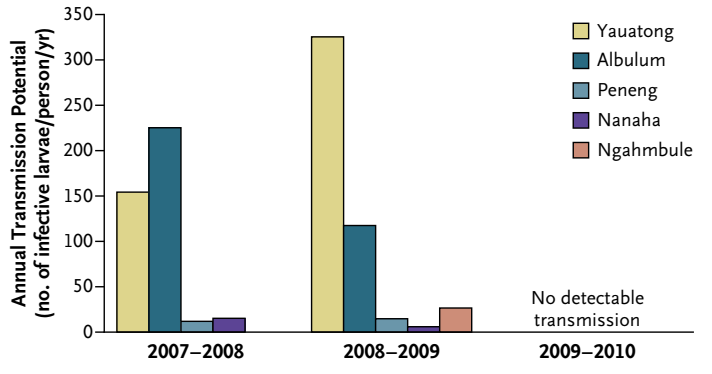

Figure 3. Annual Transmission Potential for Lymphatic Filariasis in the Five Study Villages during the 26 Months before and 11 Months after Bed-Net Distribution.

Shown are the estimated numbers of infective larvae that were inoculated per person per year during the periods of July 2007 through August 2008, September 2008 through August 2009, and September 2009 through July 2010. Insecticide-treated bed nets were distributed in the villages in August 2009.

DISCUSSION

Residents of villages in our study participated in a 5-year program of mass administration of antifilarial drugs, with 77 to 86% of eligible residents receiving such drugs annually from 1994 through 1998.[20] The prospect of the elimination of lymphatic filariasis was promising at the end of the campaign and seemed even more likely after a 2003 survey showed very few mosquitoes containing developing larvae, no mosquitoes containing infective larvae, and no children under the age of 10 years who tested positive for filarial antigen.[24] However, it is clear from observations in 2008 and 2009 that transmission was still occurring, since annual transmission potentials among the study villages ranged from 5 to 325 infective larvae that were inoculated per person per year, and the prevalence of microfilariae had significantly rebounded in three villages. Human migration may have contributed to continuing transmission and increased prevalence of microfilariae after the cessation of mass treatment.[31] The fact that worm breakpoints that were necessary for the cessation of transmission were not attained is probably of greater importance. In 1997, after four annual mass drug administrations, the prevalence of microfilariae in moderate- and high-transmission zones was 1% and 5%, respectively.[20] However, worm breakpoints for anopheline systems are estimated to be 0.75%.[11]

The introduction of insecticide-treated bed nets profoundly affected the vector population and therefore the transmission of lymphatic filariasis. A similar proportion of mosquitoes tested positive for *W. bancrofti* DNA before and after bed-net distribution. However, significantly fewer mosquitoes contained developing worms after bed-net distribution. These findings indicate that mosquitoes were imbibing microfilaremic blood, but larval development was interrupted. The use of bed nets may reduce the transmission of vectorborne diseases by shortening the life span of mosquitoes,[32] and *W. bancrofti* microfilariae require at least 13 days to develop into infective larvae in *A. punctulatus*.[33] Therefore, a slight reduction in average life span could have a major effect on the number of infective larvae. In addition, after the introduction of bed nets, most mosquitoes that fed successfully probably were feeding before residents went to bed. We saw that a greater proportion of the mosquito population was biting at earlier hours after bed nets were introduced. Because of the nocturnal periodicity of microfilaremia in Papua New Guinea, earlier biters will ingest fewer microfilariae than those biting during the time of peak blood density of microfilariae (around 1:30 a.m.) (Fig. S1 in the Supplementary Appendix).

Previous studies have shown how vector control alone can be used to reduce the prevalence of microfilariae[34] and to accelerate this decrease

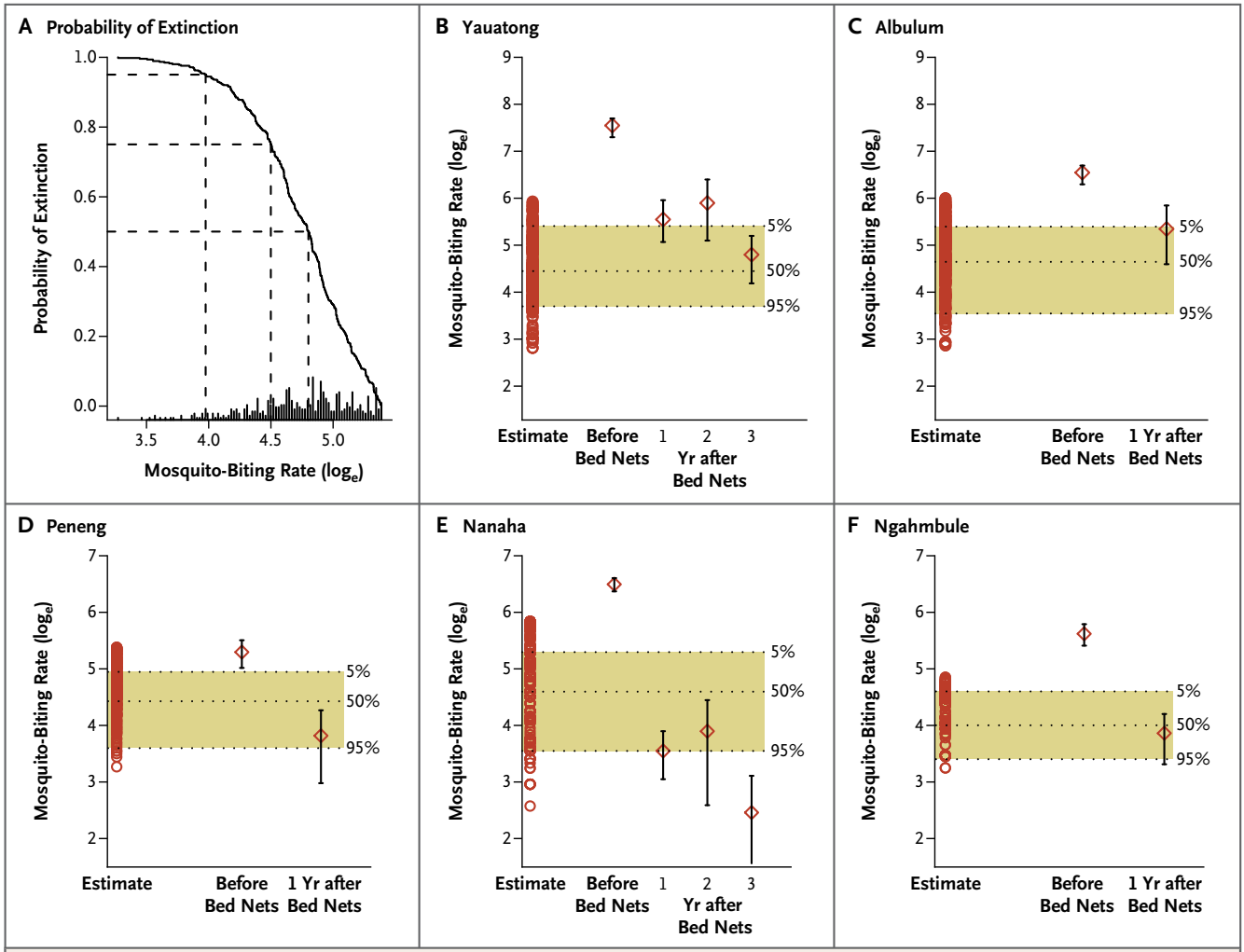

Figure 4. Probabilities of the Cessation of Transmission of Lymphatic Filariasis before and after Bed-Net Distribution on the Basis of Village-Specific Goodness of Fit with the Anopheline Transmission Model.

Panel A shows the probability of the cessation of transmission of lymphatic filariasis in the village of Peneng, according to mosquito-biting rates, which are expressed as the natural logarithm (log$_e$) on the x axis. The horizontal dashed lines show the biting thresholds associated with cessation probabilities of 50%, 75% and 95%. The bars at the bottom of the panel indicate the frequency distribution of the model-estimated biting thresholds for Peneng. Panels B through F show changes in the mosquito-biting rate and probabilities of cessation in the five study villages before the distribution of insecticide-treated bed nets and 1 or more years after bed-net distribution. (Mosquito-biting rates were available for Yauatong and Nanaha for years 2 and 3 after the distribution.) Shown are estimates (open circles) of the most likely 500 biting thresholds calculated by goodness of fit of the model with 2008 data regarding the prevalence of microfilariae, stratified according to the age of residents in the five villages in the study. The shaded bands between dashed lines denote the range and biting threshold values associated with 5%, 50%, and 95% probabilities of transmission cessation. The diamonds indicate measured biting rates, and the I bars 95% confidence intervals.

when combined with mass administration of antifilarial drugs.[35,36] However, our study quantifies the effect of the most widely implemented vector-control measure, the use of insecticide-treated bed nets. Although vector control is not currently a part of the global strategy to eliminate lymphatic filariasis, universal bed-net distribution is now used widely for malaria control-and-elimination efforts in Papua New Guinea and sub-Saharan Africa. Thus, our study highlights the importance of integrating vectorborne disease interventions. However, in order for the use of bed nets to be a sustainable strategy to eliminate lymphatic filariasis, biting rates must remain below the threshold until lymphatic-dwelling adult worms in the population die. The likelihood of transmission cessation that we observed is a snapshot of a temporally dynamic transmission

system. A small increase in biting rate with no change in human prevalence of microfilariae could quickly lead to a reestablishment of stable transmission.

Program managers wanting to determine transmission end points during elimination campaigns are met with the challenge of detecting human infection at progressively lower levels.[37] In our study, we used data on the prevalence of microfilariae before the intervention and a model of the transmission of lymphatic filariasis to quantify the probability of transmission cessation on the basis of mosquito-biting rates alone. Annual transmission potentials dropped to zero after bed-net distribution in all villages on the basis of an absence of infective larvae in blood-seeking mosquitoes, though detection of infective mosquitoes was constrained by very low vector densities. The probability of transmission cessation was more than 50% in the two moderate-transmission villages, where the prevalence of microfilariae was 3.4% or less before bed-net distribution. A similar likelihood of transmission cessation was calculated for Peneng, where the prevalence of microfilariae in 2008 was 23.7%, but the mosquito-biting rate was similar to rates in the moderate-transmission villages after bed-net distribution.

If the use of bed nets remains high and vector populations continue to be susceptible, the use of bed nets may eliminate lymphatic filariasis in areas where the reservoir of microfilariae has first been reduced by mass drug administration, as in the populations included in this study, or where preintervention endemicity is already low, such as in the Solomon Islands.[18] In high-transmission areas, the use of bed nets could work synergistically with mass drug administration by increasing the worm breakpoint to a more easily attainable level. Given the challenges of reaching 80% compliance with mass drug administration for at least 5 years,[38,39] efforts to eliminate lymphatic filariasis would greatly benefit from integrated vector management.[40]

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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Malaria Journal

RESEARCH Open Access

Malaria transmission dynamics surrounding the first nationwide long-lasting insecticidal net distribution in Papua New Guinea

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Abstract

Background: The major malaria vectors of Papua New Guinea exhibit heterogeneities in distribution, biting behaviour and malaria infection levels. Long-lasting, insecticide-treated nets (LLINs), distributed as part of the National Malaria Control Programme, are the primary intervention targeting malaria transmission. This study evaluated the impact of LLINs on anopheline density, species composition, feeding behaviour, and malaria transmission.

Methods: Mosquitoes were collected by human landing catch in 11 villages from East Sepik Province and Madang Province. Mosquitoes were collected for 3 years (1 year before distribution and 2 years after), and assayed to determine mosquito species and *Plasmodium* spp. infection prevalence. The influence of weather conditions and the presence of people and animals on biting density was determined. Determinants of biting density and sporozoite prevalence were analysed by generalized estimating equations (GEE).

Results: Mosquito biting rates and entomological inoculation rates decreased significantly after the distribution. *Plasmodium falciparum* and *P. vivax* sporozoite prevalence decreased in year 2, but increased in year 3, suggesting the likelihood of resurgence in transmission if low biting rates are not maintained. An earlier shift in the median biting time of *Anopheles punctulatus* and *An. farauti s.s.* was observed. However, this was not accompanied by an increase in the proportion of infective bites occurring before 2200 hours. A change in species composition was observed, which resulted in dominance of *An. punctulatus* in Dreikikir region, but a decrease in *An. punctulatus* in the Madang region. When controlling for village and study year, *An. farauti s.s.*, *An. koliensis* and *An. punctulatus* were equally likely to carry *P. vivax* sporozoites. However, *An. punctulatus* was significantly more likely than *An. farauti s.s.* (OR 0.14; $p = 0.007$) or *An. koliensis* (OR 0.27; $p < 0.001$) to carry *P. falciparum* sporozoites.

Conclusions: LLINs had a significant impact on malaria transmission, despite exophagic and crepuscular feeding behaviours of dominant vectors. Changes in species composition and feeding behaviour were observed, but their epidemiological significance will depend on their durability over time.

Keywords: *Anopheles punctulatus*, *Anopheles farauti*, *Anopheles kolienses*, Malaria, Papua New Guinea, Bed nets, LLIN

Background

Malaria transmission in Papua New Guinea (PNG) is highly variable across environmentally diverse zones, ranging from intense perennial transmission in the northern coastal lowlands to seasonal moderate transmission in the southern coast and unstable transmission at higher altitudes [1]. A recent survey reported weighted malaria parasite prevalence of 12 % nationwide, with substantial heterogeneity, ranging from 0 to 49.7 % [2]. Four malaria species are endemic to PNG (*Plasmodium falciparum*, *P.*

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ivax, *P. ovale*, and *P. malariae*) with the majority of infections caused by *P. falciparum* and *P. vivax* [3, 4]. The major malaria and filariasis vectors in PNG are members of the *Anopheles punctulatus* group. This group comprises 13 species, each exhibiting different degrees of exophily and anthropophily and different habitat preferences [5–8]. The five major malaria vectors in this group, due to their widespread distribution and high abundance, include *An. punctulatus*, *An. farauti s.s.*, *An. koliensis*, *An. hinesorum*, and *An. farauti* 4 [5]. In addition to the *Anopheles punctulatus* group, *An. bancroftii*, *An. longirostris*, *An. karwari*, and *An. subpictus* have been incriminated as malaria vectors.

Prior to the development of more sensitive molecular diagnostics in the 1990s [9–11], identification was restricted to the Punctulatus clade (now known to include *An. punctulatus*, *An. farauti* 4 and *An.* sp. *nr punctulatus*), Farauti clade (now known to include *An. farauti s.s., An. hinesorum, An. torresiensis, An. farauti* 5, *An. farauti* 6, and *An. farauti* 7) and *An. koliensis*. Morphological identification, based on proboscis scale patterns and the presence of a sector spot on the costal wing vein, was often unreliable in distinguishing *An. koliensis* due to variable scaling patterns, even within isofemale lines [12–15].

Although restricted by morphological identification, early studies highlight heterogeneities in habitat preference, seasonality, human blood index, and transmission potential among members of this complex. Differences were observed in the spatial distribution of species among and within villages, larval habitats and vegetation. *An. farauti s.s.* is primarily found in coastal villages with a high tolerance for breeding in brackish water. *An. farauti s.s.* and *An. hinesorum* are more commonly found in natural breeding sites, such as ground pools, while *An. punctulatus* is commonly found in areas disturbed by human activity [14, 16]. *An. punctulatus* is abundant in the hills and *An. koliensis* has a patchy distribution in lowland inland areas [17], and both exploit different larval habitats [18]. Abundance is correlated with recent rainfall, with *An. koliensis* showing greater temporal variability than *An. punctulatus* and *An. farauti s.l.* Peak outdoor biting times vary with the majority of *An. farauti s.l.* biting in the early evening and *An. koliensis* and *An. punctulatus* biting in the late night and early hours of the morning [13].

Pilot projects in PNG in the 1950s demonstrated the likelihood that DDT could successfully control malaria given the proper resources. It was not until the 1970s, through the support of United Nations Development Programme, that the DDT indoor residual spraying campaign was scaled up to cover over 50 % of the population [19]. In Madang, DDT spraying was ineffective against *An. farauti s.l.,* but very effective against *An. punctulatus* and moderately effective against *An. koliensis* [17]. In the Solomon Islands, DDT residual spraying impacted members of the complex differently [20, 21] with a stronger impact on *An. punctulatus* and *An. koliensis* than *An. farauti s.s.*

Long-lasting, insecticide-treated nets (LLINs) can be a powerful tool in reducing malaria-associated morbidity, particularly when high community coverage is achieved [22]. In PNG, where the vector population is 100 % susceptible to pyrethroids [23] LLINs remain effective for up to 5 years of in-home use [24], and are an attractive choice for malaria control. However, the tendency of the vector population to spend the majority of time outdoors [17] may render them less susceptible to indoor vector control due to low biological coverage. Furthermore, home-based interventions may have an unequal impact in areas with multiple vector species exhibiting a range of exophily and anthropophily.

The PNG Malaria Control Programme, supported by the Global Fund to Fight AIDS, Tuberculosis and Malaria, launched a countrywide, free, LLIN distribution between 2005 and 2009. PermaNet 2.0 LLINs, treated with 55 mg/cu m deltamethrin and manufactured by Vestergaard Frandsen, were distributed at a target ratio of one net per 2.5 household members. Independent household surveys reported 68.7 % of households surveyed in the Momase region (Morobe, Madang and Sepik Provinces included in this study) owned at least one LLIN (with 47 % sleeping underneath a LLIN the night before), while 95 % of households had at least one net of any type (with 74 % sleeping underneath the night before) [25]. In addition, the malaria control programme introduced rapid diagnostic tests and artemisinin-based combination therapy in 2012 [26].

The purpose of this study was to determine the impact of the LLIN distribution on mosquito abundance, species composition, peak biting times, and malaria transmission in three geographical regions of PNG with multiple, sympatric vector species.

Methods

Study villages

Eleven villages, covering three geographic regions, were included in the study (Fig. 1). Coastal villages are characterized by coconut plantations and swamps while the inland foothills (Madang and Dreikikir) are characterized by thick vegetation. Houses are typically built on stilts from bamboo or sago palm with a large open veranda. July, August and September experience lower rainfall but there is no pronounced dry season. Climate, topography and larval sites have been described elsewhere [14].

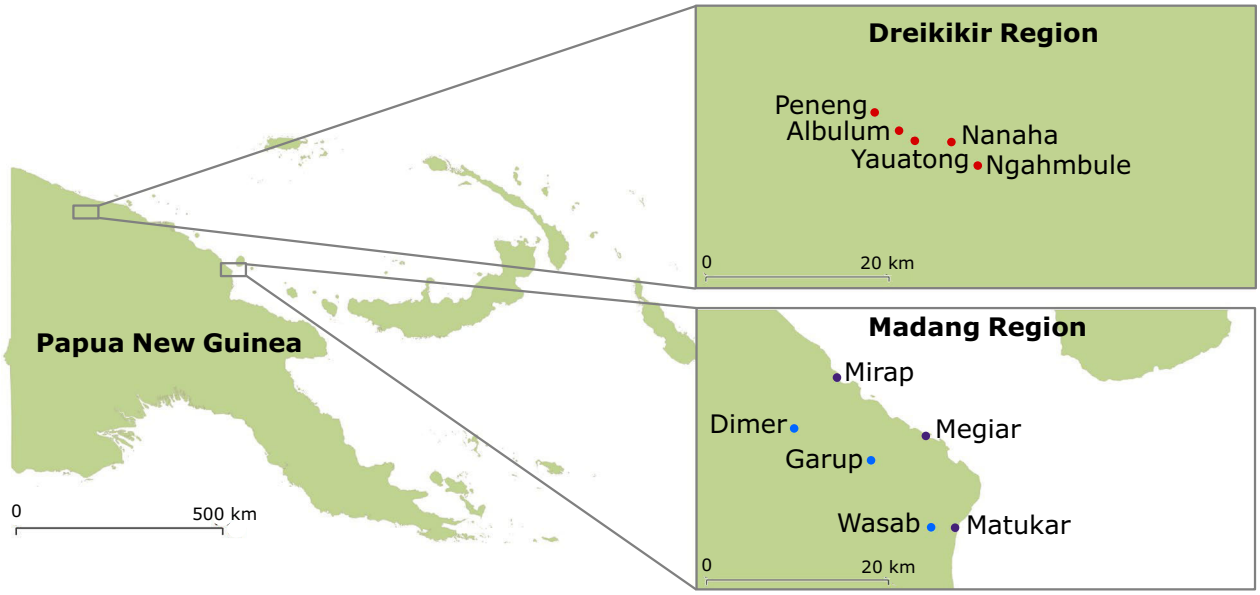

Fig. 1 Map of study villages. Madang coastal (*purple*), Madang inland (*blue*) and Dreikikir (*red*) field sites

Experimental design

Mosquitoes were collected by the outdoor human landing catch method for 1 year prior to and 1 year following the nationwide LLIN distribution. Four representative villages (Matukar, Dimer, Nanaha, Yauatong) were chosen for further surveillance throughout year 3. Collectors were trained to aspirate host-seeking mosquitoes before biting in order to minimize exposure to malaria. Pairs of collectors worked in teams, with one individual collecting all mosquitoes landing on exposed legs from 1800 to 2400 hours and the second individual collecting from 24.00 to 06.00. The collectors rotated from the early shift to the late shift on subsequent nights. Mosquitoes were stored in cups according to the date, location and hour of collection. Following morphological identification [27, 28], mosquitoes were stored dry on silica gel and returned to the laboratory for mosquito species confirmation and enzyme-linked immunosorbent assay to detect *Plasmodium* spp. circumsporozoite protein. In the Madang villages, additional data were recorded hourly by each collector, including: the number of animals and number of additional people present in the hamlet, as well as a qualitative record of wind, rainfall and cloud cover (none, light, moderate or heavy).

Collection effort

In both inland and coastal regions of Madang, the first year of mosquito collections began in August 2008 until LLINs were distributed in July and August 2009. Year 2 collections began after LLIN distribution through September 2010, and year 3 collections concluded in November 2011. In the Dreikikir region, the first year of collections began in September 2008 until LLINs were distributed in late August 2009. Year 2 collections continued until July 2010 and year 3 collections concluded in July 2011. Mosquitoes were collected monthly in every village for the first 2 years. In the third year, collections occurred only in select villages with bimonthly collections in Dimer and Matukar and quarterly collections in Nanaha and Yauatong. Details of collection effort per year are shown in Additional file 1: Table S1.

Molecular diagnostics

Mosquitoes that were morphologically identified as members of the *An. punctulatus* group were confirmed to species by PCR-restriction fragment length polymorphism of the ITS2 region (4) using either an individual leg or extracted DNA (QIAGEN, Maryland, USA). Lysates from whole mosquitoes were screened for *P. falciparum*, *P. vivax* 210 and *P. vivax* 247 circumsporozoite proteins by enzyme-linked immunosorbent assay [29].

Rainfall data

Rainfall data were collected by the PNG National Weather Service from Madang airport, 37–52 km from the Madang study villages. Although variations are to be expected between inland and coastal villages, regional rainfall records demonstrate typical seasonality and a lack of aberrant rainfall during the collection period.

Table 1 The effect of environmental variables on hourly catch of anopheline mosquitoes

Variable	Category	p value	Odds ratio	95 % CI
Wind[a]	None		1.00	
	Light	0.263	0.87	[0.69, 1.11]
	Moderate	<0.001	0.50	[0.39, 0.66]
	Heavy	<0.001	0.37	[0.27, 0.52]
Cloud[a]	None		1.00	
	Light	0.001	1.40	[1.14, 1.72]
	Moderate	0.245	0.83	[0.60, 1.14]
	Heavy	0.890	0.99	[0.83, 1.18]
Rain[a]	None		1.00	
	Light	0.563	1.06	[0.87, 1.30]
	Moderate	0.120	0.69	[0.44, 1.10]
	Heavy	0.129	0.76	[0.54, 1.08]
LLINs[b]	Absent		1.00	
	Present	<0.001	0.38	[0.29, 0.48]
People[c]		0.555		
Animal[c]		0.800		
Rain_lag[d]		0.002		

[a] Categorical variables refer to the conditions during the hour of collection

[b] Categorical variable referring to whether LLINs had been distributed

[c] Continuous variables refer to the number of alternative hosts present during the hour of collection

[d] Continuous variable referring to the amount of rainfall in the previous month

Ethical approval

This study was approved by the institutional review boards at University Hospitals Case Medical Center in Cleveland, Ohio, USA and the Institute of Medical Research and Medical Research Advisory Council of PNG.

Data analysis

Generalized estimating equations (GEE) were used to identify the determinants of mosquito abundance and infection prevalence. When the dependent variable was binary (sporozoite prevalence), a binomial distribution with a log link function was used. For count data (mosquito abundance), a Poisson distribution with log link function was used. In both cases, an exchangeable working correlation matrix was assumed. For sporozoite prevalence, separate models were constructed with either *P. falciparum* or *P. vivax* positivity as the dependent variable. Mosquito species (only *An. punctulatus* complex members were included) and year of the study (years 1, 2, 3) were covariates and village was the subject variable. For mosquito abundance, LLINs (presence or absence), rain in mm the previous month, the number of people and animals present outdoors within the compound, and the qualitative variables of rain, wind and cloud cover were included as covariates. Subject variables were village and collector, and within-subject variables were the date and hour of collection.

Species composition before and after the distribution are presented for the four intensively surveyed villages. Due to a significant association between *P. falciparum* infection and *An. punctulatus*, the proportion of this species of the total catch between year 1 and years 2 and 3 was compared by Fisher's exact test.

Median biting times were calculated based on the entire catch of a given species per village and year. Not all collected species could be PCR-confirmed, therefore only villages that showed 95 % concordance between morphological and molecular identification in a given year are presented, and the data include all morphologically identified species. Mann–Whitney U tests were performed to determine if the medians between years were the same.

Mean man-biting rates were calculated for each village and compared between years 1 and 2 or 1 and 3 (*t* test with Bonferroni correction for multiple comparisons). Entomological inoculation rates, a measure of the number of infective bites per person per year, were calculated based on the average total number of bites per person per year and the sporozoite prevalence in mosquitoes.

Results

The distribution of LLINs had a significant effect on anopheline biting density ($p < 0.001$, Table 1). In addition to the presence of LLINs, wind and rain at the time of collection were negatively associated with anopheline biting density ($p < 0.001$ and $p = 0.046$, respectively), as was rainfall from the previous month ($p = 0.002$). Cloud cover at the time of collection was positively associated with biting density ($p = 0.003$) although this relationship was limited to light cloud cover only. The number of people and animals present in the hamlet during the collection hour did not influence biting density ($p = 0.56$ and $p = 0.80$, respectively).

Mean monthly man biting rates for the Madang regions are shown in Fig. 2. In both Madang sites, biting densities peaked in September 2008 and generally declined until LLINs were distributed in August 2009. This peak corresponds with low rainfall in August following heavier rains in June (Fig. 2a). Similar patterns in rainfall were observed during all three years; however, after LLINs were distributed biting densities remained low (Fig. 2b, c).

Anopheles farauti s.s. was the dominant species collected in the coastal villages and *An. punctulatus* was dominant in inland Madang and Dreikikir (Fig. 3). *An. koliensis* was dominant in Nanaha, but only in year 1 (Fig. 3). In addition to members of the *An. punctulatus* group, *An. bancroftii*, *An. longirostris* and *An. karwari*

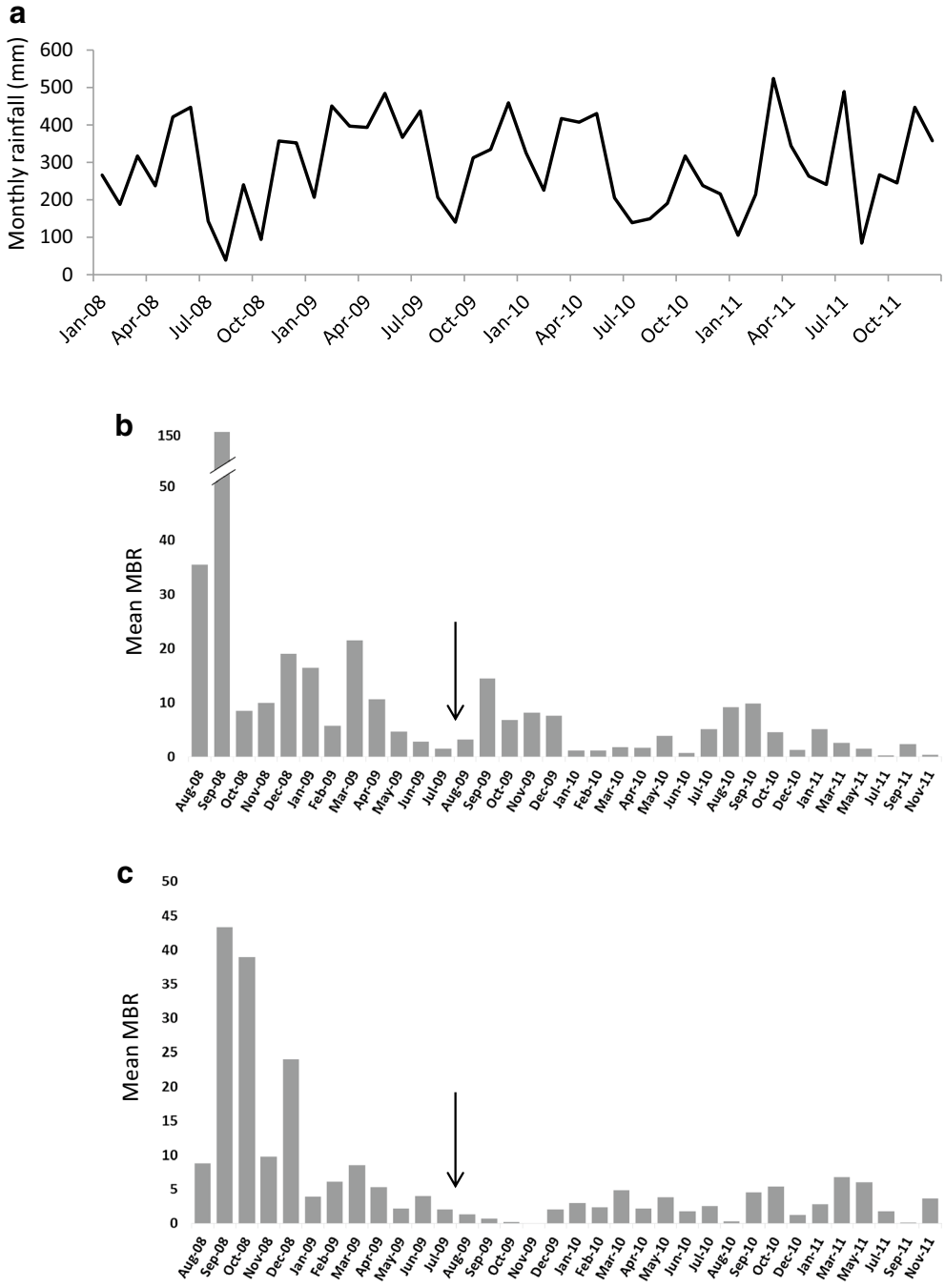

Fig. 2 Madang region monthly rainfall and biting rates. Madang airport monthly rainfall (**a**) and mean nightly man biting rates in (**b**) coastal Madang (n = 5093), (**c**) inland Madang (n = 2804). The *arrows* represent LLIN distribution dates in the community. Each village had a similar sampling effort between years 1 and 2 (August 2008–July 2010). However, in year 3 (August 2010–November 2011), only Matukar and Dimer were sampled

were collected at lower densities. An increase in the proportion of *An. punctulatus* and a decrease in the proportion of *An. farauti s.s.* were observed in both representative Madang villages (Matukar: $p = 0.0001$, Dimer: $p = 0.0001$), while an increase in the proportion of *An. punctulatus* and a decrease in the proportion of *An. koliensis* were observed at both representative Dreikikir villages (Yauatong: $p = 0.007$, Nanaha: $p = 0.0001$).

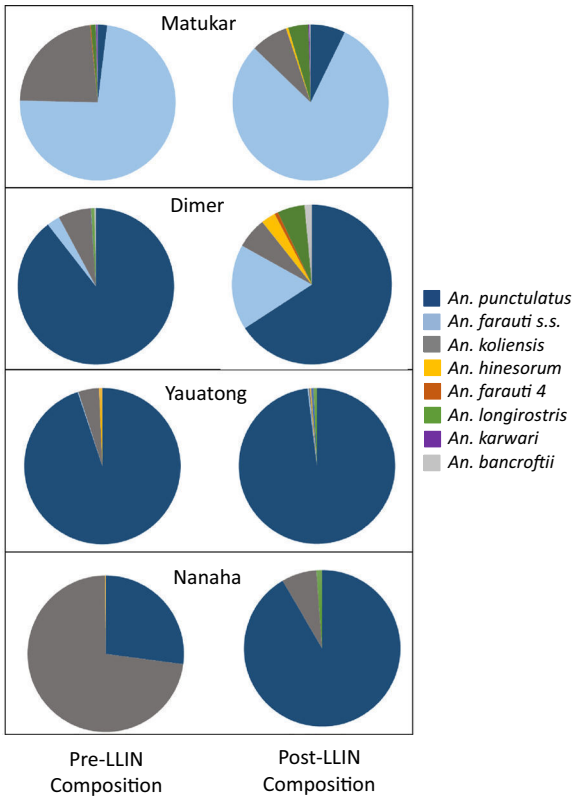

Fig. 3 Species composition before and after LLIN distribution. PCR confirmed anopheline species composition for Matukar (n = 1182 pre-LLIN, n = 430 post-LLIN), Dimer (n = 1417 pre-LLIN, n = 272 post-LLIN), Yauatong (n = 722 pre-LLIN, n = 324 post-LLIN), Nanaha (n = 738 pre-LLIN, n = 83 post-LLIN)

Anopheles farauti s.s. had a tendency for earlier biting compared to *An. punctulatus* and *An. koliensis* with 38, 16 and 15 % biting before 22.00, respectively. Significant shifts were observed in median biting time after the LLIN distribution for *An. punctulatus* and *An. farauti s.s.* (Fig. 4), with the exception of *An. punctulatus* from Ngahmbule village. Despite this shift, there was no statistically significant difference in the proportion of infective bites occurring before 22.00 pre-LLIN (42.9 %) compared to after LLINs (35 %, Fisher's exact p = 0.56).

Individual village level analyses showed a significant reduction in mean annual man biting rates in eight of the 11 villages following the LLIN distribution (Fig. 5; p < 0.003). The three remaining villages had the lowest pre-intervention biting rates per region and they exhibited reductions that were non-significant (Megiar p = 0.11, Garup p = 0.13, Peneng p = 0.009). A reduction in annual entomological inoculation rate (Fig. 5) was observed in all villages except Garup.

GEE analysis indicated that, when controlling for village and mosquito species, the chance of a mosquito carrying sporozoites in the second year was significantly lower than the pre-intervention period for both *P. falciparum* (OR 0.12; 95 % CI 0.03, 0.51; p = 0.004) and *P. vivax* (OR 0.17; 95 % CI 0.08, 0.36; p < 0.001). In the third year, the odds of sporozoite positivity rebounded for both *P. vivax* (OR 0.49; 95 % CI 0.27, 0.87; p = 0.015) and *P. falciparum* (OR 0.74; 95 % CI 0.44, 1.25; p = 0.261), although the probability of carrying *P. vivax* sporozoites was still less than in the pre-intervention period (Additional file 2: Table S2). When controlling for village and study year, *An. farauti s.s.*, *An. koliensis* and *An. punctulatus* were equally likely to carry *P. vivax* sporozoites. However, *An. punctulatus* was significantly more likely than *An. farauti s.s.* (OR 0.14; 95 % CI 0.03, 0.58; p = 0.007) or *An. koliensis* (OR 0.27; 95 % CI 0.13, 0.57; p < 0.001) to carry *P. falciparum* sporozoites (Fig. 6).

Discussion

Mosquito populations have been extensively studied in Madang and East Sepik Provinces, where *P. falciparum* and *P. vivax* are both highly endemic. In Dreikikir, pre-LLIN sporozoite rates and man biting rates were similar to those observed in the late 1950s [30]. In Madang Province, sporozoite rates are similar to previous studies [13, 31] but observed man biting rates were lower than historically reported [31, 32].

Overall, man biting rates dropped significantly in the year following LLIN distribution and remained low in representative villages through the third year. Even though members of the *An. punctulatus* group do not exhibit preferences for endophagy [17], LLINs may still have a large communal impact if LLIN coverage and usage is high [33]. LLIN usage in study sites was higher than regionally reported [25]. A survey 3 months following the distribution in Dreikikir revealed that 79 % had slept under a LLIN the previous night [34]. A separate survey conducted in Madang study sites 3 years after the distribution revealed that 79 % had slept under a LLIN in Mirap and 54 % had slept under a LLIN in Wasab (JBK, unpublished). A separate study has shown that individual LLIN use and high community LLIN coverage were independently and strongly associated with reduced odds of malaria infection in PNG [2].

Although biting rates decreased, a trend towards earlier biting was observed in *An. punctulatus* and *An. farauti s.s.* Changes in biting behaviour were previously reported in *An. farauti* and *An. koliensis*, in a nearby village, following distribution of insecticide-treated nets in 1985 [35]. In PNG, this shift could be indicative of behavioural flexibility or the impact of LLINs on population age

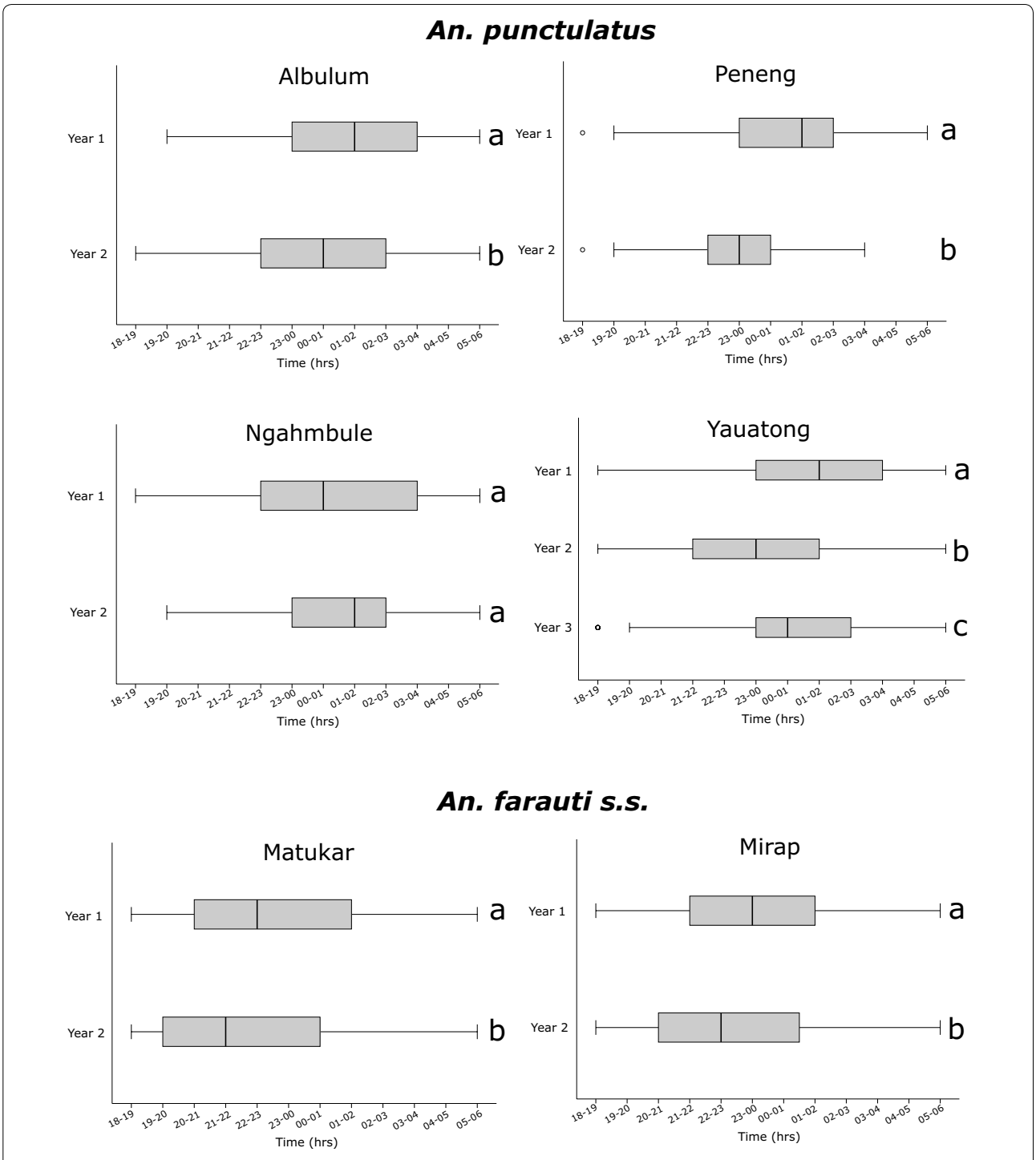

Fig. 4 Biting time before and after LLIN distribution. *Boxes* indicate first to third quartile and median hours of biting activity. *Whiskers* represent fifth to 95th percentiles. Year 1 was before LLIN distribution and years 2 and 3 were after. *Boxes* carrying the same letter were not statistically different (Bonferroni adjusted alpha = 0.007)) when comparing median biting times using Mann–Whitney U tests. Albulum year 1 n = 874, year 2 n = 383; Peneng year 1 n = 715, year 2 = 103; Ngahmbule year 1 n = 596, year 2 = 100; Yauatong year 1 n = 2818, year 2 n = 672, year 3 n = 464; Matukar year 1 n = 2187, year 2 n = 187; Mirap year 1 n = 1191, year 2 n = 328

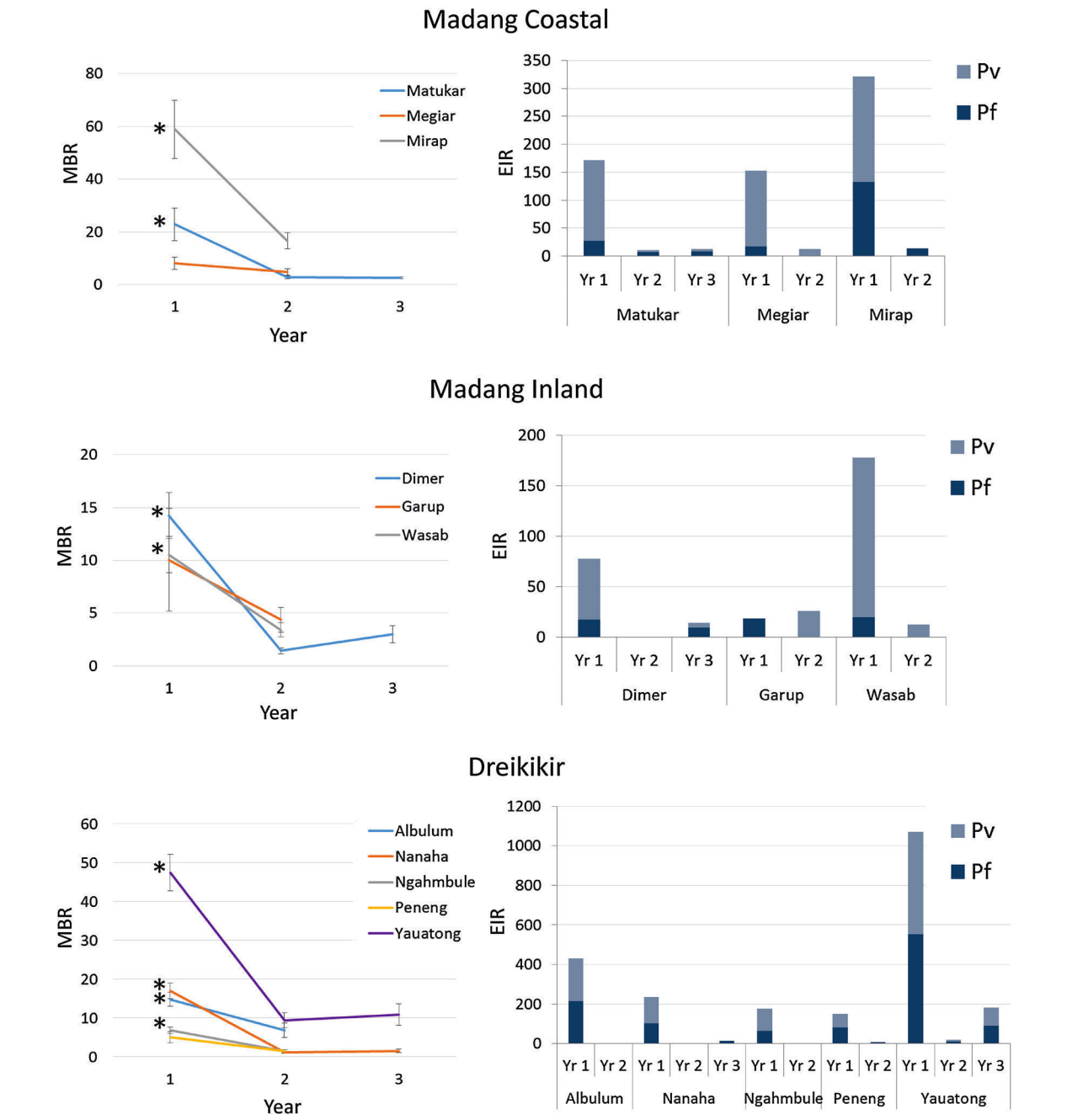

Fig. 5 Man biting rate and entomological inoculation rate. Panels on the left show mean nightly biting rate (± SEM) in villages from each region. *Asterisk* indicates villages which experienced a significant reduction between years 1 (pre-LLIN) and 2 or 3 (post LLIN) (Bonferonni adjusted alpha = 0.003). Panels on the right show entomological inoculation rate (infective bites/person/year) with *P. falciparum* and *P. vivax* in year 1 (pre-LLIN), 2 and 3 (post-LLIN) for each region

structure; it has been observed that nulliparous females bite earlier than parous females [36]. The former scenario has been suggested in an *An. farauti s.l.* population in the Solomon Islands following a DDT spraying campaign [20]. Additional behavioural and genetic studies could elucidate whether selection is occurring in populations in this study. In the latter scenario, early biting patterns would not be accompanied by increased exposure even if biting densities returned to pre-control levels. No increase in the proportion of infective bites occurring before 22.00 in this study was observed, although sample sizes were limited. Continued surveillance can determine

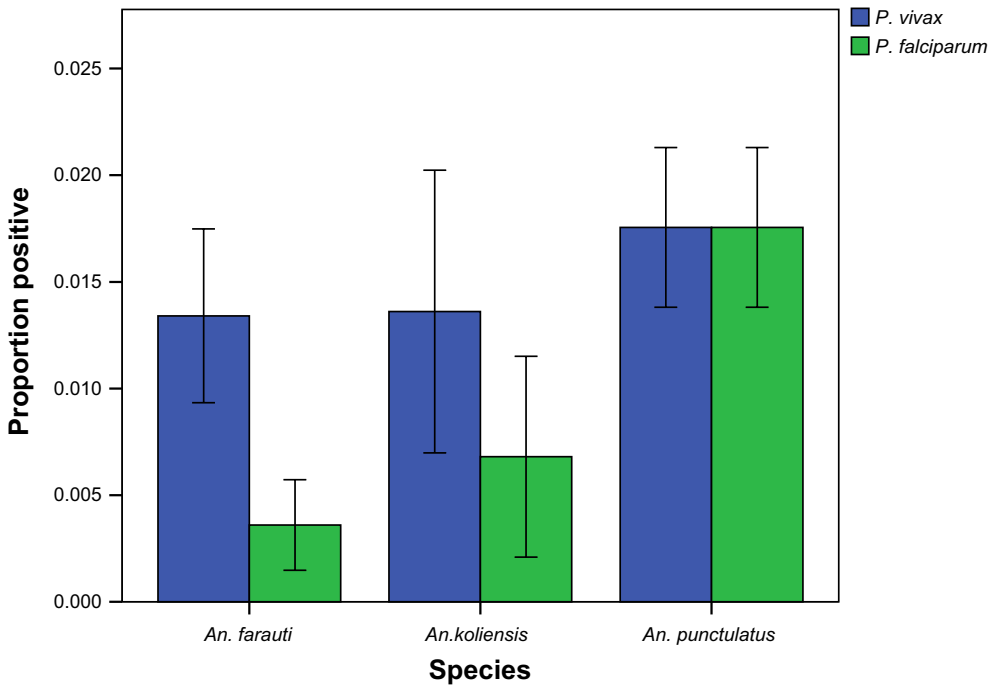

Fig. 6 *Plasmodium* spp. sporozoite prevalence in dominant anophelines

if the observed shifts in biting times are epidemiologically significant, and whether they will limit the protective effect of LLINs.

Differences in species composition were observed after LLIN distribution. However, there was not a consistent trend across villages towards species dominance. For example, in the Madang villages, *An. farauti s.s.* increased in proportion over *An. punctulatus* while in Dreikikir, *An. punctulatus* increased in proportion over *An. koliensis.* It is likely that some species will be more resilient to this intervention than others, and changes in species composition will depend on behaviour and physiology of the entire population. The observed pattern may be attributed to a greater impact of LLINs on the later biting species in the community. The observed increase in the proportion of *An. punctulatus* could be epidemiologically significant given the close association between this species and *P. falciparum* transmission. Further work is needed to determine whether these changes in species composition are durable, and whether they are associated with malaria prevalence.

While LLINs were very effective in reducing man biting rate and entomological inoculation rate in the three regions, sporozoite prevalence did not remain low. The observed increase in sporozoite prevalence between years 2 and 3 may be attributable to a low sample size, a challenge in post-intervention evaluations. However, it is clear that residual malaria transmission is still occurring, and that any rebound in mosquito density will be accompanied by an increase in transmission intensity. In both Madang regions, *P. falciparum* sporozoite rate was higher in year 3 than in year 1. This result is surprising because vector control is expected to have a greater impact on *P. falciparum* than *P. vivax*, due in part to the longer extrinsic incubation period for *P. falciparum* and the role of dormant hypnozoites in *P. vivax* transmission. These higher infection rates in year 3 may be influenced by low mosquito numbers and further work to determine long-term trends is needed.

Conclusions

This study has demonstrated a strong community impact of LLINs on exophagic and early biting vector populations. Success may be attributable to high LLIN ownership as well as a portion of the vector population that continues host seeking during the time when people are indoors. Individual malaria exposure will be affected by house construction, personal bed net use and other human behavioural patterns. Although LLINs had a clear impact on vector populations, the impact on sporozoite prevalence did not extend to year 3. Given the plasticity of mosquito behaviour and natural fluctuations in vector densities, continued surveillance is needed to determine changes in the effectiveness of LLINs.

Additional file

Additional file 1: Table S1. Annual collection effort and total anophelines from each village.

Additional file 1: Table S2. Prevalence of *Plasmodium* spp. in wild anophelines.

Authors' contributions

PAZ, LJR, IM, MH, JWK, and PS conceived and designed the study; GK, JBK, LJR, and EKT supervised the field activities and conducted the research. LJR and EKT analysed the data; LJR wrote and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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Mosquito Behavior Change After Distribution of Bednets Results in Decreased Protection Against Malaria Exposure

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Background. Behavioral resilience in mosquitoes poses a significant challenge to mosquito control. Although behavior changes in anopheline vectors have been reported over the last decade, there are no empirical data to suggest they compromise the efficacy of vector control in reducing malaria transmission.

Methods. In this study, we quantified human exposure to both bites and infective bites of a major malaria vector in Papua New Guinea over the course of 4 years surrounding nationwide bednet distribution. We also quantified malaria infection prevalence in the human population during the same time period.

Results. We observed a shift in mosquito biting to earlier hours of the evening, before individuals are indoors and protected by bednets, followed by a return to preintervention biting rates. As a result, net users and non–net users experienced higher levels of transmission than before the intervention. The personal protection provided by a bednet decreased over the study period and was lowest in the adult population, who may be an important reservoir for transmission. Malaria prevalence decreased in only 1 of 3 study villages after the distribution.

Discussion. This study highlights the necessity of validating and deploying vector control measures targeting outdoor exposure to control and eliminate malaria.

Keywords. Infectious Disease Vectors; Mosquito Control; Mosquito behavior; Insecticide-Treated Bednets; Malaria.

In the past 2 decades, global efforts to reduce the burden of malaria have intensified. Since 2000, the primary strategy to limit transmission has been the distribution of insecticide-treated bednets (ITNs). Recent estimates suggest that in the last 15 years, ITNs have been responsible for preventing 68% of the 663 million cases that have been averted in sub–Saharan Africa due to increased malaria control efforts [1]. However, it is well recognized that strategies solely targeting endophagic, anthropophagic, and endophilic vectors may not be sufficient to control and eliminate malaria [2]. This is particularly true outside Africa where vectors exhibit greater behavioral plasticity. Control efforts can result in shifts in vector behavior and/or species composition such that the post-intervention vector community is less likely to come in contact with insecticide [3–9].

Studies to accurately quantify exposure to bites and the true protective efficacy of long-lasting insecticidal nets (LLINs) [10] have revealed that the vast majority of exposure still occurs inside, when people can be protected by an LLIN [11]. Thus, despite shifts to outdoor feeding [4, 5, 12] and changes in biting times [13], the personal protection provided by LLINs remains high (>80%). In areas outside of sub–Saharan Africa where vectors bite earlier and outside, LLINs can still reduce transmission through the combined effect of frequent blood-feeding and a homogenous host-seeking phenotype [14, 15]. Regardless, in some settings, evidence suggests that these behavioral changes are decreasing the personal protection against bites offered by an LLIN [16], a worrying prospect for malaria control and elimination in these areas.

It is well established that residual malaria transmission (transmission that remains despite universal coverage of effective interventions [2]), can be intense. However, it is currently unknown whether the interventions that are deployed against malaria vectors have the ability to increase residual transmission intensity through shifts in behavior or how shifts in behavior may impact human infection prevalence. Modeling suggests that the presence of behavioral resistance could dramatically increase transmission, perhaps more so than physiological resistance [8], which is currently poised to create a public health disaster if not confronted immediately. Behavioral resistance could have catastrophic consequences for the sustainability of currently available vector control methods, especially in areas outside of sub–Saharan Africa characterized by outdoor transmission [17, 18]. In this study, we estimate the exposure

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to infective bites experienced by children and adults in Papua New Guinea (PNG) before and after a nationwide LLIN distribution. In addition, we quantified malaria infection prevalence in the mosquito and human populations. We show that following the intervention, there was a shift in the biting behavior of the major malaria vector, *Anopheles farauti* 4. This caused the protective efficacy of LLINs to decrease, and the ability of nets to control malaria in this situation was compromised.

METHODS

Mosquito Collection

Longitudinal monitoring of mosquito abundance was performed by outdoor human landing catch in Kokofine (−5.69029, 145.4801) and Mauno (−5.65079, 145.493) villages in Madang Province of PNG. These villages sit 4.6 km apart in the Ramu River valley. Trained collectors sat outside a house with their pant legs rolled up. They collected host-seeking mosquitoes that landed on their legs with an aspirator, and stored all captured mosquitoes in cups according to hour of collection. One collector worked from 6 PM to 12 AM, and another worked from 12 AM to 6 AM. The collectors switched shifts on sequential collection nights. Two houses were sampled each night, and collections were performed for 6 consecutive nights. The first collections in both villages occurred in December 2008, 1 month before LLINs were distributed in January 2009 [19]. Subsequent collections occurred in November of 2009 and September of 2010. In 2011, collections were performed in March, July, and November, but no significant seasonal variation was observed in either village (in both mean biting rates and infection rates), so results from these 3 months were pooled in subsequent analyses. The species of mosquito was confirmed by polymerase chain reaction–restriction fragment length polymorphism of the ITS2 region [20] using either an individual leg or extracted DNA. Lysates from whole mosquitoes were screened for *Plasmodium falciparum*, *Plasmodium vivax* 210, and *P. vivax* 247 circumsporozoite proteins by enzyme-linked immunosorbent assay [21] in pools of 5 mosquitoes each.

To estimate the proportion of bites experienced inside and outside, additional collections were performed in June 2011. During this month, indoor landing catches were performed simultaneously with the outdoor landing catches at 1 chosen household per night for 6 consecutive nights. The degree of endophagy is presented as the proportion of mosquitoes collected by indoor landing catches out of the paired total.

Human Behavior

Human movement inside and outside was quantified as part of a national household survey and questionnaire [22]. Heads of household were asked what time individuals in the house went inside, what time they went to bed, and how old they were. Kokofine and Mauno were not included as part of this household survey, but data concerning human movement were similar across the lowland regions of the country. Therefore, patterns of movement and bed times in the Momase region were used in this analysis.

Human Infection Prevalence

Human infection prevalence was measured in February and March of 2008, 2009, and 2011 in Mauno, Kokofine, and Kesowai (−5.79683, 145.62299) villages. The methods used in this household survey have already been described [19]. Briefly, a finger prick blood sample was taken from consenting individuals aged >5 months old from 30–35 randomly selected households in each village. Stained blood slides were double-read by trained microscopists at the PNG Institute of Medical Research.

Data Analysis

Nightly biting rates were compared between years using a 1-way analysis of variance and Tukey's test for post-hoc comparisons. Median biting times, 1st and 3rd quartiles, and 95% confidence intervals were calculated based on the entire catch per village and year. Kruskal–Wallis tests with pairwise comparisons were performed to determine whether the distribution of biting times between years was the same. Sporozoite prevalence was calculated as minimum prevalence, where positive pools were assumed to only have 1 positive mosquito. Prevalence for each year was calculated by dividing the total number of positive pools (with either *P. falciparum* or *P. vivax*) by the total number of mosquitoes in all pools analyzed. Prevalence was compared between years with chi-square tests. At 10 PM, 90% of individuals were inside, so this time point was chosen to compare sporozoite prevalence in early biting mosquitoes using a chi-square test. Four indices of exposure and protection were estimated: exposure to bites (either for a net user [B_p] or a non–net user [B_u]), the proportion of exposure occurring indoors (π_i), the true protection against mosquito bites (P^*), and the true protection against infective bites (P^{*f}). Estimates of exposure to bites for net users and nonusers were calculated as published previously [10] with 2 modifications. First, because paired indoor and outdoor landing catches were not performed during the entire study period, indoor hourly biting rates were estimated by first calculating the hourly proportions biting inside and outside during the paired collections. Hourly proportions were then multiplied by hourly outdoor biting rates to estimate hourly indoor biting rates. Second, estimates of indoor exposure for net users was refined by accounting for the period after individuals moved inside and before they went to bed. The estimate of exposure for a net user (B_p) was therefore:

$$B_P = \sum_{t=1}^{24} \left[B_{o,t}\left(1 - I_t\right) + B_{i,t}\left(I_t - S_t\right) + B_{i,t} S_t \left(1 - P\right) \right],$$

where $B_{o,t}$ is the outdoor biting rate at time t, I_t is the proportion of individuals inside at time t, $B_{i,t}$ is the indoor biting rate at time t, S_t is the proportion of individuals sleeping at time t, and

P is the protection provided by nets, which is assumed to be 0.968 [23]. Similar modifications were made to the calculation of B_u, P*, and π_i.

Exposure to infective bites was estimated by first calculating the hourly infection rate N_t. Exposure to infective bites for a net-user was therefore

$$F_p = \sum_{t=1}^{24} B_{p,t} N_t,$$

and for a non-user

$$F_u = \sum_{t=1}^{24} B_{u,t} N_t.$$

The personal protection against infective bites (P^{*f}) provided by an LLIN was

$$P^{*f} = 1 - \frac{F_p}{F_u}.$$

B_u was compared across years in each village using Kruskal–Wallis tests with pairwise comparisons. π_i and P* were compared among age groups and years using generalized linear mixed models with a binomial distribution, village as a subject, year by age group as the fixed effect, and household nested within date as a random effect. For each dependent variable, a dataset was constructed using the formulas described herein (or derivatives of) to estimate exposure values for each household and date combination. All statistical analyses were performed with SPSS 22.

Prevalence of malaria positivity was compared between years within each village using chi-square tests.

Ethical Approval and Informed Consent

Informed consent was obtained from all participants or their parent/guardian for those aged <16 years. This study was approved by the institutional review board at the Papua New Guinea Institute of Medical Research (protocol 0933) and the Medical Research Advisory Council of PNG (protocol 10.12).

Results

Biting Rates

Over the course of 4 years (2008–2011), 41 757 anopheline mosquitoes were captured by 138 outdoor human landing catch collections. More than 99% (n = 41 407) were identified as *An. farauti sensu lato*. The remaining mosquitoes were identified as *An. koliensis* (n = 157), *An. punctulatus* (n = 122), *An. longirostris* (n = 69), and *An. karwari* (n = 2). All 4267 of the *An. farauti s.l.* mosquitoes that were confirmed by polymerase chain reaction were *An. farauti* 4. The nightly biting rate significantly decreased 1 year after LLINs were distributed in both villages (from 560 to 212 bites/person/night in Kokofine, $P = .001$; and from 156 to 37 bites/person/night in Mauno, $P < .001$). In Kokofine, nightly biting rates increased significantly in 2010 (to 374 bites/person/night) and remained at that level in 2011 (418 bites/person/night). In Mauno, nightly biting rates remained low but did increase significantly between 2010 (4 bites/person/night) and 2011 (66 bites/person/night, $P < .001$).

Host-Seeking Behavior

The median outdoor biting time in both villages occurred significantly earlier after the distribution of LLINs (Figure 1A and 1B). In Kokofine, the median biting time was 11 PM–12AM in 2008 and was 1 hour earlier in 2009. Although the value of median biting time returned to 11 PM–12AM in 2010–2011, there was still a significant shift from the preintervention value due to the change in bite time distribution, with more mosquitoes biting earlier than the median time after LLIN distribution. In Mauno, the median biting time was 12 PM–1 AM in 2008, and shifted 2 hours earlier (10 PM –11 PM) in 2009. In 2010 and 2011, the median biting time remained at 10 PM–11 PM, but the distribution of bites continued to shift even earlier. In both villages, the hour of maximum biting density was 10 PM–11 PM in 2008, and 8 PM–9 PM in 2011 (Figure 1C and 1D). The degree of endophagy remained relatively consistent throughout the hours of the night, with 16.5% of overall bites occurring inside (Supplementary Figure 1).

Mosquito Infection Prevalence

Sporozoite prevalence remained consistent across all 4 years in Mauno but increased significantly in 2011 in Kokofine (Figure 2). Mosquito infection prevalence ranged from 0% to 0.54% in Kokofine and 0% to 0.42% in Mauno. The proportion of infective bites occurring before 10 PM was not different between years.

Human Sleeping Behavior

Movement of people indoors occurred slightly earlier in the highland than in the lowland regions of PNG. Sleeping patterns were similar across the 4 main geographical regions (Supplementary Figure 2).

In Momase, where Kokofine and Mauno are located, data were disaggregated by sex and age. Adolescent and adult males had later patterns of activity than females, and younger individuals went inside and went to bed earlier than older individuals (Figure 3A and 3B). The proportion of individuals sleeping under an LLIN did not exceed 0.71 among any age group at any time of night, with males aged 15–19 years the least protected at 0.54 (Figure 3C). A separate study in this village reported net usage at 91% in 2012 (J. Keven, L. Reimer, M. Katusele, G. Koimbu, R. Vinit, N. Vincent, E. Thomsen, D. Foran, P. Zimmerman, and E. Walker, submitted).

Exposure

After a significant decrease in exposure between 2008 and 2009 in both villages ($P < .001$ for Kokofine and Mauno),

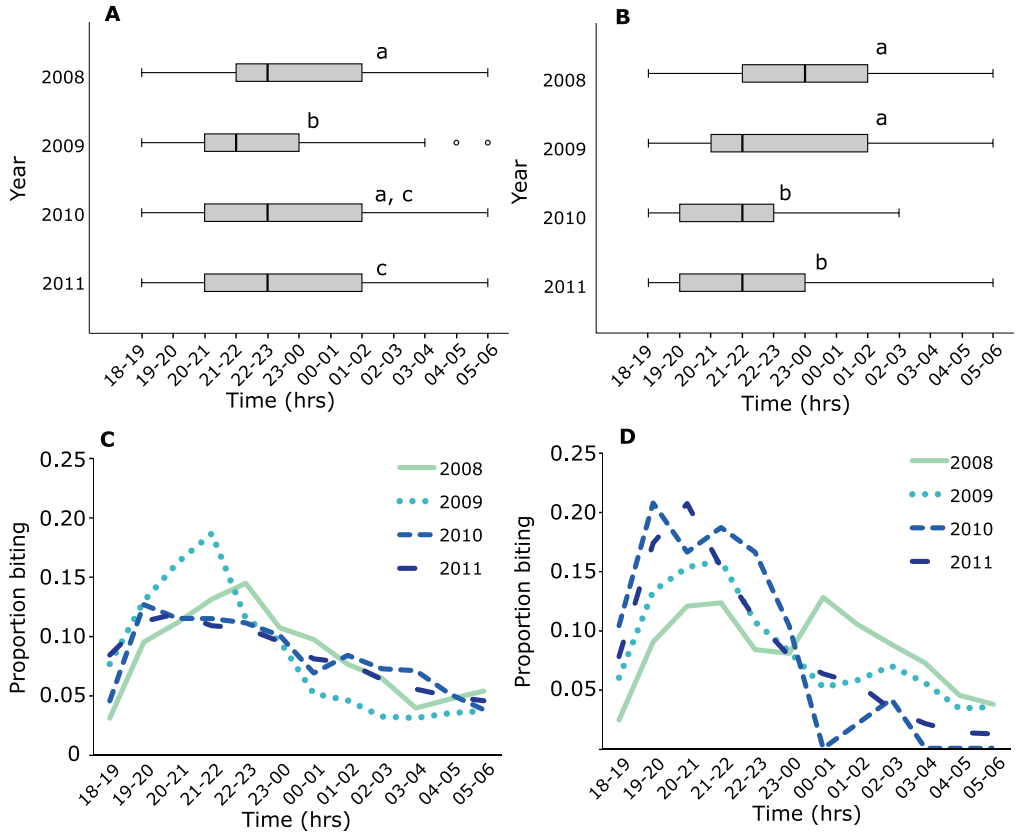

Figure 1. Median outdoor biting times with 1st and 3rd quartiles (boxes) and 95th percentiles (whiskers) in Kokofine (*A*) and Mauno (*B*) villages of Papua New Guinea before (2008) and after (2009–2011) a long-lasting insecticidal net distribution. Years not sharing the same letter indicate significantly different medians using a Kruskal–Wallis test with pairwise comparisons. The proportion of bites occurring at each hour in Kokofine (*C*) and Mauno (*D*) are presented as well.

there was subsequently a significant increase from 2009 to 2011 in Kokofine and from 2010 to 2011 in Mauno (Figure 4). Shifts to earlier bite exposure were observed in both villages (Supplementary Figure 3). Within each year, the estimated proportion of exposure occurring inside (π_i) and the protective efficacy against bites (P*) was significantly greater in younger age groups ($P < .001$ for all years). Within each age group, there was a decrease in π_i and P* after LLINs were distributed, and

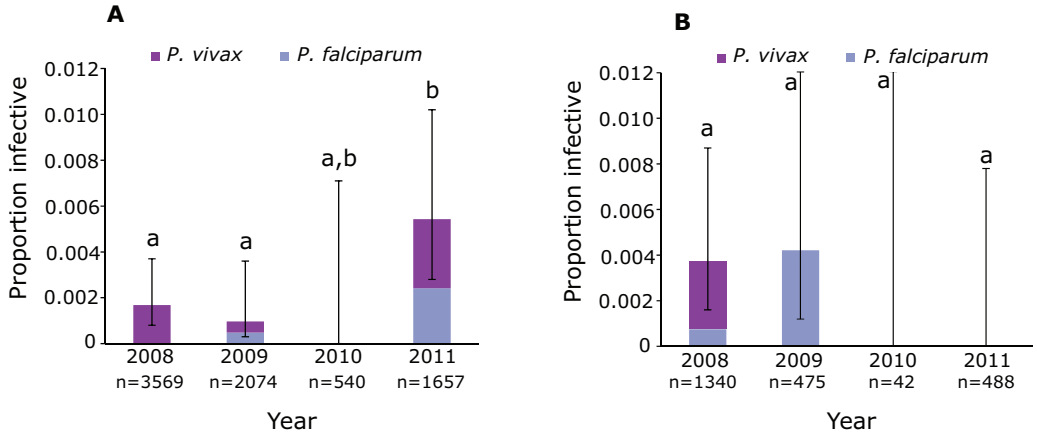

Figure 2. Sporozoite prevalence for *Plasmodium falciparum* and *Plasmodium vivax* in *An. farauti* 4 in Kokofine (*A*) and Mauno (*B*) villages of Papua New Guinea before (2008) and after (2009–2011) a long-lasting insecticidal net distribution. Sample sizes are indicated below each year. Bars not sharing the same letter indicate significant differences using chi-square tests.

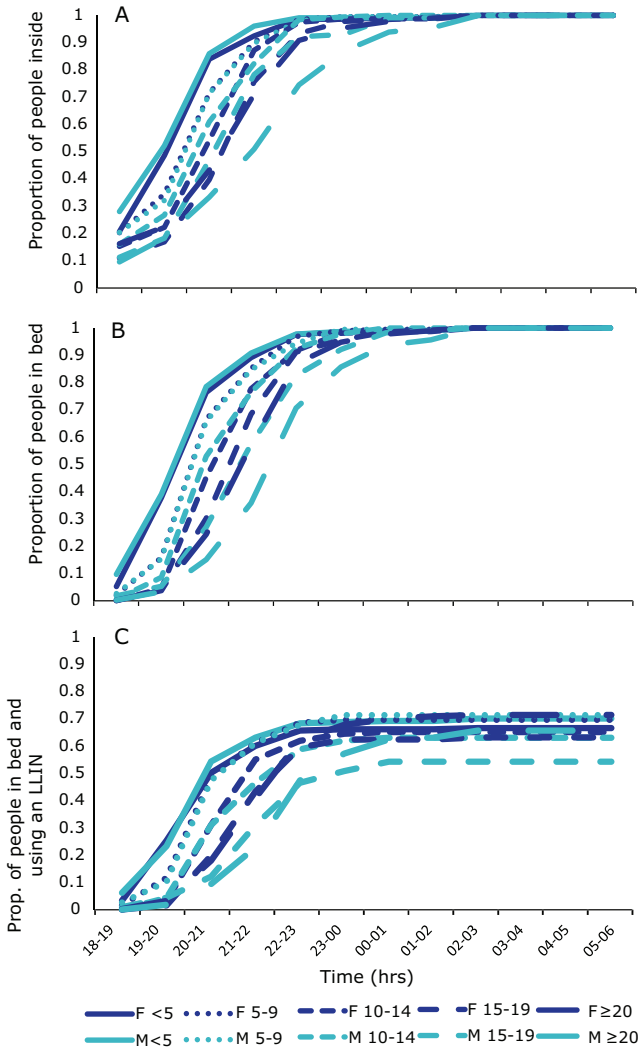

Figure 3. Proportion of males (M) and females (F) from each age group inside (*A*), in bed (*B*), and in bed under a long-lasting insecticidal net (LLIN) (*C*) from 6 PM to 6 AM.

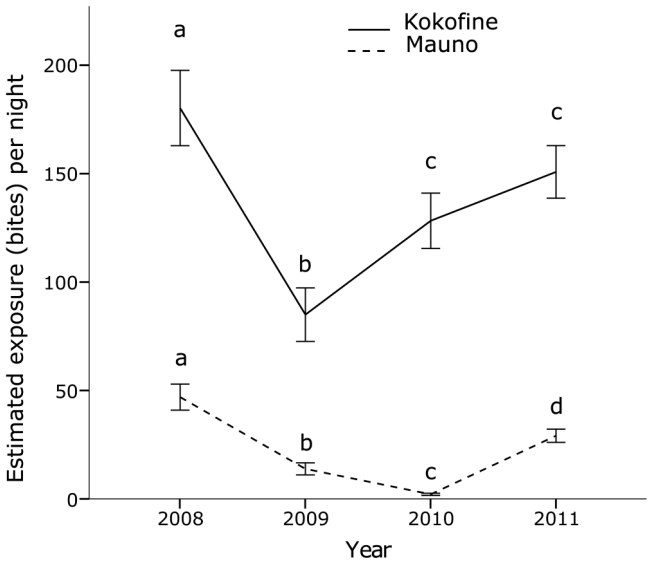

Figure 4. Total estimated exposure to bites for a non–net user before bednets (2008) and after bednets (2009–2011) in Kokofine and Mauno. Years sharing the same letters were not statistically different using a Kruskal–Wallis test with pairwise comparisons.

the decrease was more pronounced in younger age groups (Figure 5; for π_i: <5, $P < .001$; 5–9, $P = .002$; 10–14, $P = .004$; 15–19, $P = .008$; >20, $P = .03$; and for P*: <5, $P = .001$; 5–9, $P = .003$; 10–14, $P = .006$; 15–19, $P = .01$; >20, $P = .02$).

In Kokofine, the rebound in biting rates coupled with high sporozoite prevalence after LLIN distribution (in 2011) allowed us to quantify exposure to infective bites (Figure 6). In 2008, most exposure to infective bites occurred after 9 PM. In 2011, the majority of infective bites occurred during the first hour of collection, between 6 PM and 7 PM. In children aged <5 years, many infective bites would have been prevented by using a net in 2008; however, the protective efficacy of LLINs against infective bites (P^{*f}) decreased drastically in 2011 because these bites were occurring before this age group went to bed. In adults aged >20 years, a similar but less pronounced decrease in P^{*f} was observed, primarily because this age group was always outside when infective mosquitoes were seeking a host. In 2008, P^{*f} was 0.78 in those aged <5 years and 0.30 in those aged >20 years. In 2011, P^{*f} had decreased to 0.30 and 0.15 in both groups, respectively.

Human Infection Prevalence

Data from the 2008 and 2009 malaria prevalence surveys have been published elsewhere [19] and are presented in greater detail here for context. Only Mauno showed a consistent and significant decrease in malaria prevalence across the 3 surveys. In Kokofine, there was no significant change in overall infection prevalence, and in Kesowai there was a nearly significant increase in prevalence from 2008 to 2011 ($P = .058$) (Figure 7).

DISCUSSION

Our data show a clear and dramatic reduction in mosquito abundance in the first year after the LLIN distribution in both villages. However, a resurgence in mosquito abundance and exposure was documented between 2 and 3 years after intervention, coupled with a shift to significantly earlier biting. Based on the interaction between mosquito and human behavior, the protective efficacy of LLINs decreased during this resurgence, as more exposure to malaria vectors occurred before individuals were protected with a net. Besides behavioral resilience [24], 2 other factors may have contributed to the documented resurgence in mosquito abundance. First, physiological resistance to insecticides has also been shown to reduce intervention efficacy [25]. However, resistance is absent in members of the *An. punctulatus* group (the species group to which *An. farauti* 4 belongs) in PNG [26], and susceptibility has been confirmed from the Sausi region post-LLIN distribution (M. Katusele, unpublished data). This demonstrates

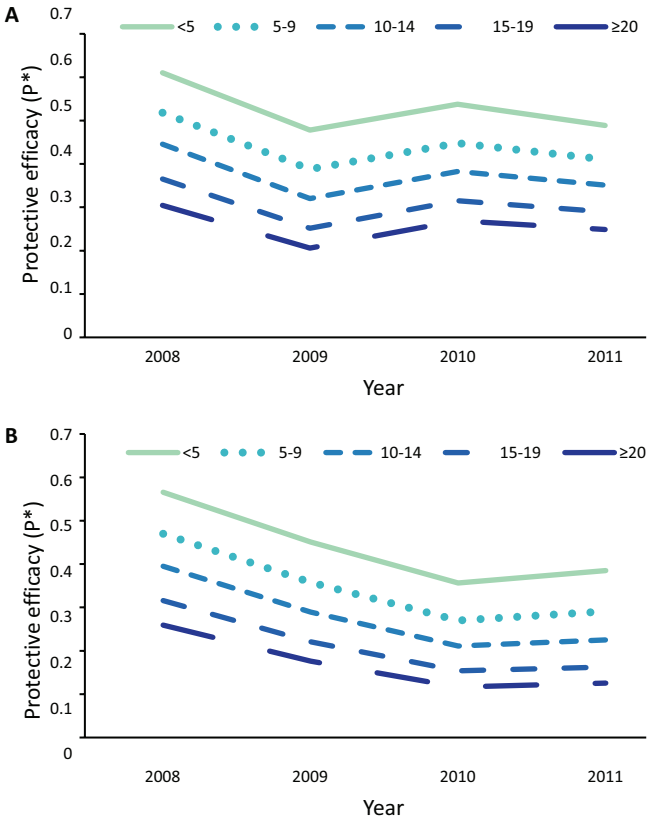

Figure 5. Protective efficacy (P*) by age group in Kokofine (*A*) and Mauno (*B*).

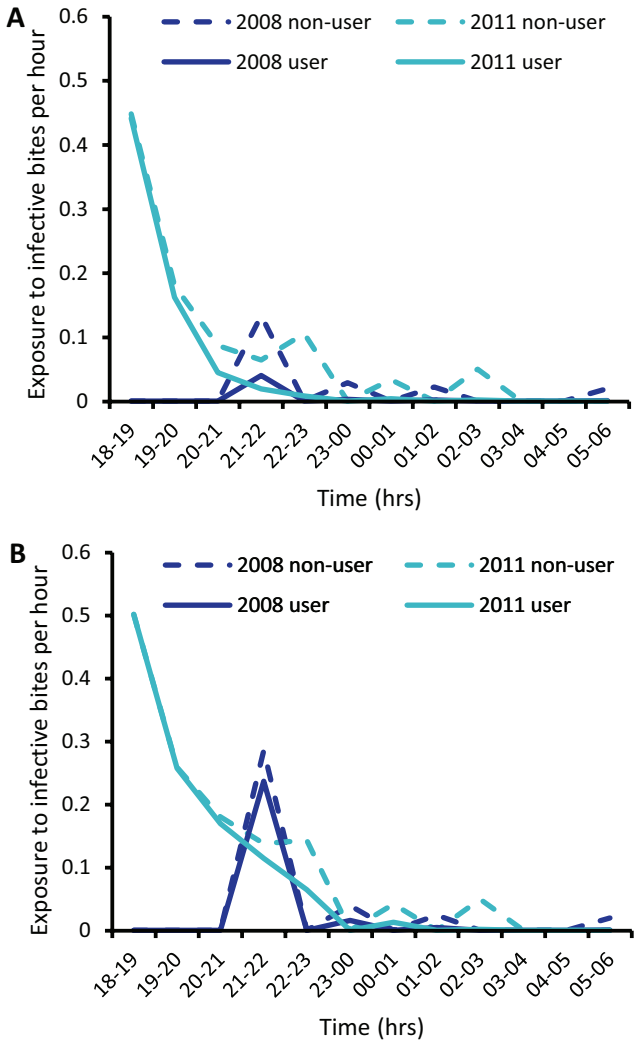

Figure 6. Estimated exposure to infective bites in children aged <5 years (*A*) and adults aged >20 years (*B*) in Kokofine. Exposure was estimated separately for bednet users and nonusers at the time of the distribution (2008) and 3 years later.

that behavioral resilience may compromise intervention efficacy in a mosquito population that is fully susceptible to insecticide. Second, changes in bednet usage over time could limit the community effect of nets; however, usage increased over the study period. Furthermore, used nets from local communities retain the insecticidal effect against *An. farauti* for 5 years [27].

This is the first study to quantify human malaria infection prevalence in the context of shifting mosquito behaviors after an LLIN distribution. Malaria prevalence in humans decreased in only 1 of 3 villages, the village with the lowest biting rates, demonstrating the limited efficacy of nets to prevent disease transmission. Although the shifts in biting times would contribute to the limited epidemiological impact of the intervention, there are other factors that may have also played a role. Artemisinin combination therapy was only rolled out to the Sausi health center in the last quarter of 2011, which means that the population may have been receiving inadequate treatment. Treatment failures with the previous combination of chloroquine and sulphadoxine-pyrimethamine reached 18.5% in children with *P. falciparum* in PNG [28]. Migration of individuals into the study communities from areas of higher malaria burden may have also been a factor. Regardless, shifts in biting times have been documented in other mosquito populations in PNG [9]. As such, it will be important to continually monitor the epidemiological impact of LLINs in other areas where changes in mosquito behavior have been observed.

This is also the first study to quantify age-stratified exposure to bites of malaria vectors by taking into account the behaviors and sleeping patterns of each age group. Both the proportion of indoor bite exposure (π_i) and the protective efficacy of LLINs against bites (P*) is greater in younger age groups, due to earlier sleeping patterns. This results in protection by an LLIN for a greater proportion of the entire period of exposure, which is a positive finding because this group is the most at risk for severe disease [29]. In contrast, the protective efficacy in adults is quite low (approximately 0.35 in both villages at the time of distribution) due to their greater outdoor activity patterns in the early hours of the night. Adults will continue to act as a reservoir of gametocytes, and LLINs may therefore have little impact on transmission reduction

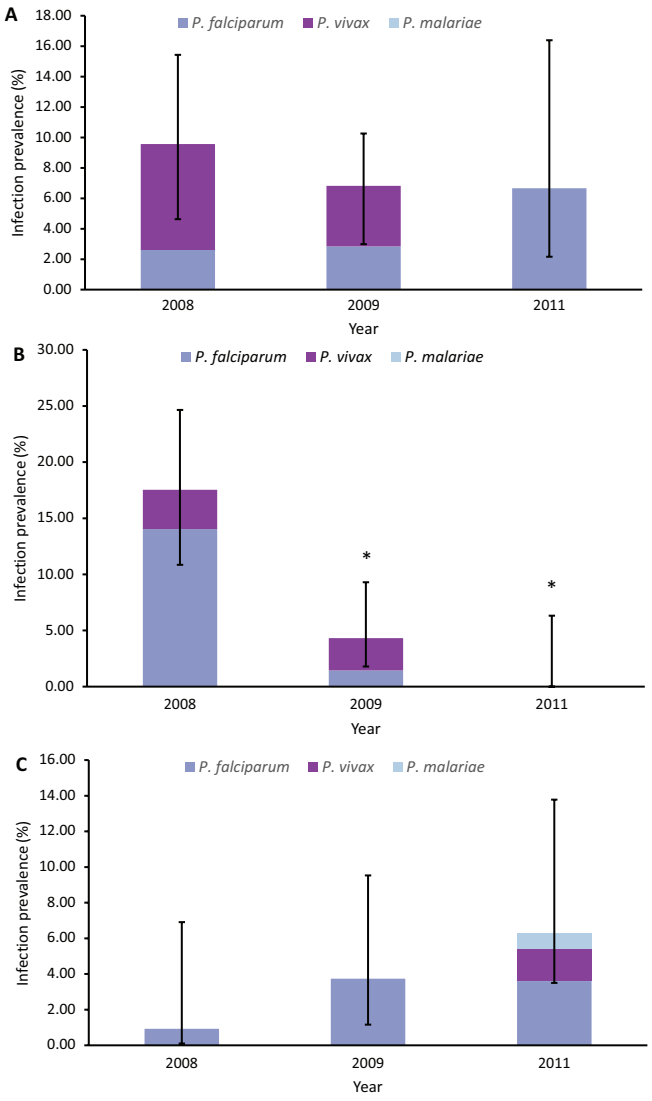

Figure 7. Human infection prevalence as detected by microscopy in Kokofine (*A*), Mauno (*B*), and Kesowai (*C*) villages before (2008) and after (2009 and 2011) a nationwide LLIN distribution in PNG. * indicates a significant change from 2008 ($P < .05$) using a chi-square test.

at the community level. This level of personal protection against bites is similar to that seen in other areas of the South Pacific [16].

The true protective efficacy of nets (against bites [P*] and infective bites [P^{*f}]) decreased in both villages after LLINs were distributed. The reduced efficacy due to shifts in host-seeking times is a phenomenon that has been observed in other studies. In *An. funestus*, a shift to early morning feeding in southern Benin did not result in compromised efficacy because P* remained >80% [13]. In *An. farauti s.s.*, a shift to early evening feeding in the Solomon Islands did reduce P* [16]. However, individual mosquitoes showed no fidelity to biting time or location, and malaria burden continued to decline [14]. The authors hypothesized that over the course of several gonotrophic cycles, the likelihood of exposure to an LLIN before the end of the *Plasmodium* extrinsic incubation period still remained high [15]. Unlike the studies described above, our study suggests that the shift to early evening feeding in *An. farauti* 4 is epidemiologically significant—the estimate of the annual entomological inoculation rate in Kokofine was 827 infective bites per person per year in 2011, more than double the estimate of 343 in 2008. In addition, our analysis indicated that individuals were less protected from infective bites in 2011 than they would have been in 2008 due to the time infective mosquitoes were collected.

The underlying mechanism for the shift in biting times observed in this vector population is currently unknown. Biting behavior in anophelines appears to be a heritable trait because shifts in host-seeking behavior in the Solomon Islands during the dichlorodiphenyltrichloroethane spray campaign of the 1970s [3] remain to this day [16]. However, additional evidence for population-level selection for behavioral resistance is lacking [7]. Today, populations of *An. farauti s.s.* in the Solomon Islands are homogenous in their host-seeking behavior: subpopulations exhibiting different feeding preferences do not exist [14]. In addition, the genes responsible for the variation in feeding behaviors in malaria vectors have yet to be identified [30]. Additional hypotheses for the mechanism include associative learning [24] and delayed host-seeking due to unsuccessful attempts the previous night [31].

The sampling scheme used in this study had several limitations. First, human landing catches were not performed before 6 PM. This may have resulted in significant undersampling of the biting population after LLIN distribution. Second, the ratio of indoor to outdoor biting rates was measured during 1 collection period after LLIN distribution. The high degree of exophagy measured here is consistent with reports of *An. farauti* 4 in neighboring Papua, Indonesia [18], as well as other members of the *An. farauti* complex in the Solomon Islands [32]. Decreases in endophagy have been observed following indoor interventions [3, 4], which we are unable to capture in our study design. If early biting mosquitoes were undersampled or if the population experienced a shift in endophagy, the analysis would have underestimated the decreases in personal protection. Third, collections were performed in 2 weeks in 2008, 2009, and 2010 and 6 weeks in 2011, which may have highlighted week-to-week variation and obscured long-term trends.

Indoor interventions such as LLINs have contributed greatly to the reduction in malaria transmission over the last 15 years [1] and continue to provide significant community protection even in cases where shifts in biting behavior have been observed [6, 11, 12, 14, 15]. Our study highlights that in an area of high vector density and intense year-round transmission, shifts in biting behavior can have detrimental impacts on the personal protection provided by LLINs as well as community-wide transmission. Shifts to earlier biting after the bednet distribution resulted in greater exposure to infective bites, in net users

and nonusers alike. The intervention achieved a reduction in malaria prevalence in only 1 of 3 villages studied despite high usage rates and net efficacy. Additional tools targeting outdoor and early biting mosquitoes will be necessary to control malaria and prevent a resurgence of transmission.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflict of interests. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Clinical Infectious Diseases

MAJOR ARTICLE

Efficacy, Safety, and Pharmacokinetics of Coadministered Diethylcarbamazine, Albendazole, and Ivermectin for Treatment of Bancroftian Filariasis

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Background. Available treatments for lymphatic filariasis (LF) are limited in their longterm clearance of microfilaria from the blood. The safety and efficacy of a single-dose triple-drug therapy of the antifilarial drugs diethylcarbamazine (DEC), ivermectin (IVM), and albendazole (ALB) for LF are unknown.

Methods. We performed a pilot study to test the efficacy, safety, and pharmacokinetics of single-dose DEC, IVM, and ALB in *Wuchereria bancrofti*-infected Papua New Guineans. Adults were randomized into 2 treatment arms, DEC 6 mg/kg + ALB 400 mg (N = 12) or DEC 6 mg/kg + ALB 400 mg + IVM 200 μg/kg (N = 12), and monitored for microfilaria, parasite antigenemia, adverse events (AEs), and serum drug levels.

Results. Triple-drug therapy induced >2-log reductions in microfilaria levels at 36 and 168 hours after treatment compared with approximately 1-log reduction with 2 drugs. All 12 individuals who received 3 drugs were microfilaria negative 1 year after treatment, whereas 11 of 12 individuals in the 2-drug regimen were microfilaria positive. In 6 participants followed 2 years after treatment, those who received 3 drugs remained microfilaria negative. AEs, particularly fever, myalgias, pruritus, and proteinuria/hematuria, occurred in 83% vs 50% of those receiving triple-drug compared to 2-drug treatment respectively ($P = .021$); all resolved within 7 days after treatment. No serious AEs were observed in either group. There was no significant effect of IVM on DEC or ALB drug levels.

Conclusions. Triple-drug therapy is safe and more effective than DEC + ALB for Bancroftian filariasis and has the potential to accelerate elimination of lymphatic filariasis.

Clinical Trials Registration. NCT01975441.

Keywords. lymphatic filariasis; chemotherapy; diethylcarbamazine; albendazole; ivermectin.

Wuchereria bancrofti is a mosquito-transmitted, chronically disabling nematode infection that causes lymphedema, elephantiasis, and hydroceles. *Wuchereria bancrofti* is endemic in 73 countries, infecting approximately 100 million people [1]. The World Health Organization has targeted lymphatic filariasis (LF) for global elimination by 2020 [2]. Since there is no drug that reliably kills or sterilizes adult filarial worms, the focus of the Global Programme to Eliminate Lymphatic Filariasis (GPELF) has been on the use of mass drug administration (MDA) to reduce the source of microfilaria in endemic populations and thereby interrupt transmission. The current MDA strategy is to provide repeated, annual doses of albendazole (ALB) with either diethylcarbamazine (DEC) or ivermectin (IVM) for the lifespan of adult worms (typically 5–7 years) [3]. Mathematical models of LF transmission suggest that the most potent drug combination currently recommended (annual DEC + ALB) will require high compliance (>70%) with MDA for 5–7 years in order to achieve elimination targets, particularly in areas with moderate to high endemicity [4]. Clinical trials have shown that a single dose of this combination clears microfilaria in only approximately 25% of participants at 12 months [5–8]. Drug regimens with better activity against microfilaria would prevent new infections that would otherwise have to be treated in later years. This could significantly improve the chances for eliminating LF in resource poor settings.

Addition of the potent microfilaricide IVM to DEC + ALB may improve microfilaria clearance and provide a more long-lasting effect than the widely used 2-drug regimen [7–9]. Prior community studies have shown that MDA with IVM plus DEC was more effective for reducing microfilaria rates than DEC alone [10]. A single dose of IVM completely cleared microfilaria in 35% of participants and reduced the geometric mean microfilaria level by >98% at 1 year; 2 years after a single treatment, 20% of participants remained microfilaria negative, and geometric mean microfilaria levels

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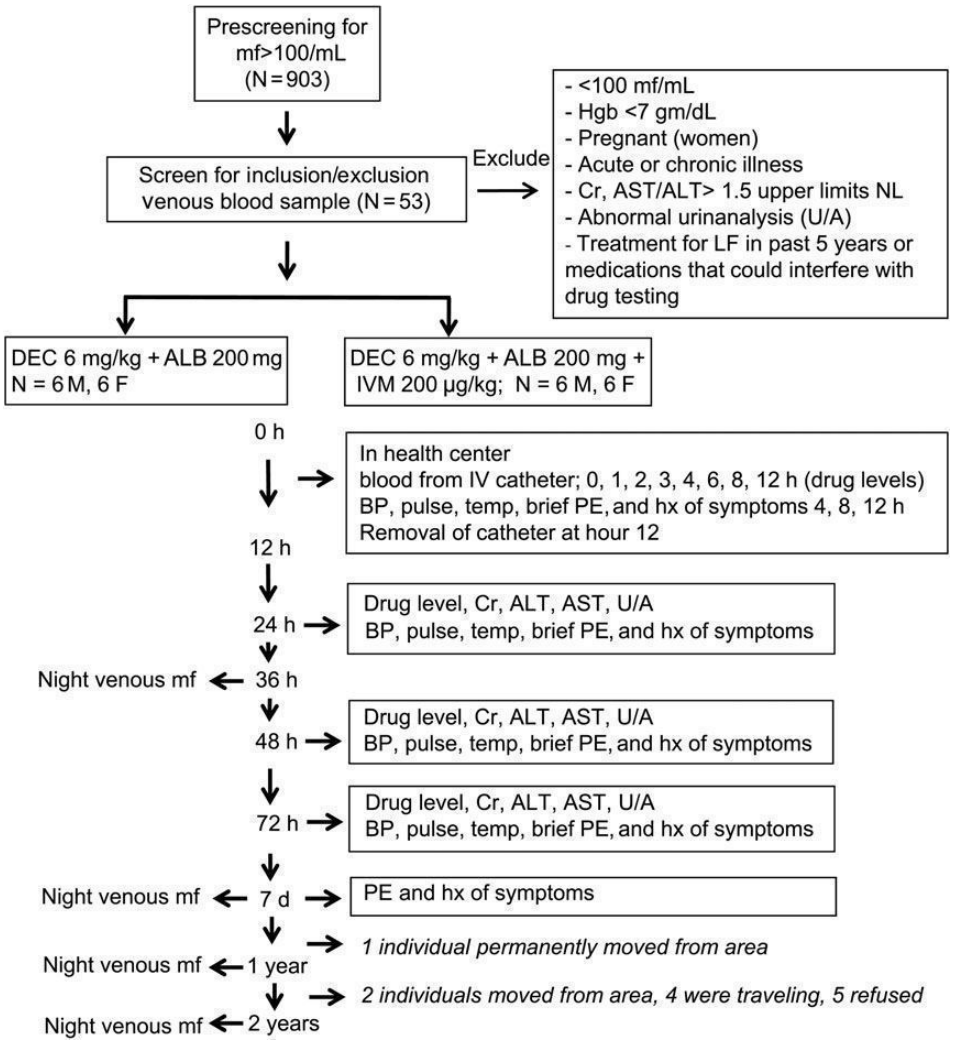

Figure 1. Study design. Abbreviations: ALB, albendazole; ALT, alanine transaminase; AST, aspartate transaminase; BP, blood pressure; Cr, creatinine; DEC, diethylcarbamazine; F, female; Hgb, hemoglobin; hx, history; IV, intravenous; IVM, ivermectin; LF, lymphatic filariasis; M, male; mf, microfilaria; NL, normal; PE, physical examination; U/A, urinanalysis.

remained reduced by >90% [11]. Similar but less profound effects were observed for single-dose IVM in a trial conducted in Tanzania [12]. It is possible that simultaneous treatment with IVM + DEC + ALB could reduce the number of rounds of MDA required to reach LF elimination, and this could have a transformative impact on the 60% of LF-exposed populations that reside outside of sub-Saharan Africa.

Since the tolerability and potential drug interactions of this 3-drug combination have never been investigated, we undertook a pilot study to compare the safety, tolerability, pharmacokinetics, and efficacy of IVM + DEC + ALB vs DEC + ALB in individuals with Bancroftian filariasis.

METHODS

Study Location

Participants were recruited from the villages of Tau 1 and 2 (3.6718 S,142.7254 E), Dreikikir District, in East Sepik Province, Papua New Guinea, with microfilaria rates of 10%–44%. No prior treatment for LF has ever been given in these 2 communities. All participants where hospitalized at the Dreikikir Health Center for the initial days following treatment.

Selection of Study Participants and Study Design

Study inclusion and exclusion criteria were as follows: aged 18–60 years, >100 microfilaria /mL, no prior antifilarial medications, or free from any acute or chronic illnesses. If inclusion and exclusion criteria were fulfilled, enrolled individuals had a 1-mL venous night blood sample (after 2200 hours) and serum biochemistries measured to ensure that alanine transaminase (ALT), aspartate transaminase (AST), and creatinine were <1.5 times normal; hemoglobin levels were >7 gm/dL; and there was no significant urine proteinuria, hematuria, or glucosuria by dipstick measurement 1 to 2 weeks prior to hospitalization (Figure 1). A total of 903 individuals were prescreened, 53 were enrolled, and 24 met the final inclusion and exclusion

criteria and agreed to participate in the study. One individual withdrew 72 hours after treatment and 2 individuals failed to return on day 7 but did have blood drawn 1 year later. Eleven individuals were unavailable at the 2-year follow-up (Figure 1). This was a single-blinded, parallel-group, randomized study with 2 treatment arms. Participants were stratified by sex and randomly assigned to 1 of 2 treatment groups: DEC 6 mg/kg + ALB 400 mg or DEC 6 mg/kg + IVM 200 µg/kg + ALB 400 mg. A blood sample was taken to establish baseline ALT, AST, and creatinine levels immediately prior to treatment. Participants were given a breakfast of peanut butter and biscuits prior to observed administration of drugs. Blood draws were performed at 0, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, and 72 hours and 7 days following experimental drug treatment (Figure 1).

Parasitological, Biochemical, and Drug Testing

Parasitemia levels were assessed by passing 1 mL of anticoagulated, nocturnally collected blood through a 5-µm polycarbonate filter (EMD Millipore Corp., Billerica, Massachusetts), washed with filtered water, placed on glass slides, dried, and stained with Giemsa. Microfilariae were counted by microscopy. Antigen levels were measured using the Og4C3 mAb–based assay [13]. Plasma for drug levels was stored at −80°C. Evaluation of AST, ALT, and creatinine levels was performed using a Vitros DT-60 II biochemistry analyzer (Ortho Clinical Diagnostics, Rochester, New York). Glucose, blood, protein, nitrites, and leukocytes were measured with a urine dipstick (Multistix 10 SG, Bayer/Seimens, Malvern, Pennsylvania), with severity graded according to the manufacturer's protocol. Hemoglobin levels were evaluated on a HemoCue Hb 201+ machine (HemoCue Inc. Cypress, California).

All pharmacokinetic analyses were performed at the University of Iowa's College of Pharmacy. The concentration of DEC was determined using high-performance liquid chromatography with mass spectrometric detection [14]. IVM concentrations were determined using a previously published extraction methodology [15]. The linear range of the calibration curve was 0.20–400 ng/mL from 0.20 mL plasma.

Clinical Evaluation and Adverse Events Recording

The night prior to drug dosing, a thorough clinical assessment was performed. Circumference of all limbs was recorded at each time point. Scrotal abnormalities were classified as edema, hernia, or hydrocele, and any hydroceles graded as smaller or larger than a fist.

Objective adverse events (AEs) were based on serial physical examinations, biochemical evaluations, and urinalysis every 4 hours for the first 12 hours then at 24, 48, and 72 hours and 7 days after treatment (Figure 1). AEs were defined as any one of the following: any increase in ALT, AST, or creatinine measurement >1.5 times the upper limit of the reference range; tympanic temperature >37.8°C; increase in lymph node tenderness, swelling, or pain from baseline; or increase in proteinuria or hematuria from baseline based on urine dipstick measurement. A scoring system was used for proteinuria and hematuria based on severity and duration of the abnormal dipstick measurement. First hematuria (or proteinuria) was scored as negative (0), mild (1+), moderate (2+), and severe (3+) with a negative assigned a score of 0, mild a score of 1, moderate a score of 2, and severe a score of 3. If an abnormal urine dipstick measurement was observed on just 1 testing day post-treatment (eg, at 24, 48, and 72 hours and day 7 post-treatment), a score of 1 was given. If the urine dipstick measurement remained abnormal for 2 days, a score of 2 was given to a maximum of 4 if the urinalysis remained abnormal for the 4 testing time points. A final score for each treatment group was determined by adding the sum of all these values for the12 individuals in each group.

Subjective AEs were assessed by asking participants about symptoms that they may have experienced after taking the medication that either increased in severity or was experienced for the first time following treatment. Participants were asked to categorize any symptoms as mild (does not interfere with daily activity), moderate (interferes with daily activity), or severe (warrants hospital admission).

Informed Consent and Regulatory Monitoring

Institutional review boards at the University Hospitals Case Medical Center, Cleveland, Ohio, the Papua New Guinea Institute of Medical Research, and the Medical Research Advisory Committee of Papua New Guinea approved study protocols and documents. The trial was registered at ClinicalTrials.gov (NCT01975441).

Data Treatment and Analyses

The study was powered to look at drug interactions and AEs, with changes in microfilaria levels as a secondary outcome. Objective and subjective AEs were compared separately using Mann–Whitney–Wilcoxon rank sum test. Differences in microfilaremia levels were assessed using student *t* test of log-transformed data. A general linear model was used to examine the independent effects of treatment and microfilaremia levels on the frequency of AEs.

For each participant, pharmacokinetic parameters were estimated by plotting the plasma concentrations (of DEC, ALB, albendazole sulfoxide [ALBSO], albendazole sulfone [$ALBSO_2$], or IVM) vs time using noncompartmental analysis (WinNonlin v5.0, Pharsight Corporation, Cary, North Carolina). The maximum plasma concentration (C_{max}) and time of maximum concentration (T_{max}) were observed directly from the concentration–time curve. The half-life, area under the curve, and drug–drug interactions were calculated as previously described [14–17] and are included in the Supplementary Materials.

RESULTS

Population Characteristics and Impact of Treatment on Infection Levels

Before treatment study participants in the 2 treatment groups had similar infection intensities, age, weight, and hemoglobin levels (Table 1). A single dose of DEC + ALB + IVM resulted in almost total elimination of microfilaria at 36 hours and 7

Table 1. Population Characteristics and Pretreatment Infection Levels

Treatment Group	N	Male/ Female	Geometric Mean Microfilaremia (microfilaria /mL) ± 95% CI (range)	Filarial Antigen (unit/mL) Geometric Mean ± 95% CI	Median Age, y (range)	Mean Weight, kg ± SD	Mean Hemoglobin, g/dL ± SD
Diethylcarbamazine + albendazole + ivermectin	12	6/6	1558 ± 2322 (209–13 776)	3881 ± 1227	30 (19–59)	53 ± 9	11.2 ± 1.3
Diethylcarbamazine + albendazole	12	6/6	1857 ± 2191 (133–13 333)	3347 ± 1018	28 (19–50)	49 ± 8	11.0 ± 1.2

Abbreviations: CI, confidence interval; SD, standard deviation.

days after treatment, and no participant was microfilaremic 12 months after treatment (Figure 2). By contrast, a single dose of DEC + ALB resulted in less dramatic reductions in microfilaria levels at 36 hours and 7 days, and 10 of 11 participants remained microfilaremic at the 12-month time point. Twelve participants who had not moved away and agreed to have an additional night blood sample drawn (by chance, 6 in each treatment group) were examined for microfilaria levels 2 years following treatment (Table 2). All 6 individuals who received the single 3-drug treatment remained amicrofilaremic at 2 years ($P = .047$, compared with those receiving 2 drugs; Table 2). DEC + ALB + IVM also resulted in greater decreases in filarial antigen levels compared with DEC + ALB at 12 months (Figure 3). All participants in both treatment groups remained antigen positive at 12 months and at 2 years.

Adverse Events Following Treatment

Objective and subjective AEs were mild to moderate in severity and were experienced by participants in both treatment groups (Table 3). The first AEs started 4 hours after treatment, typically they were subjective findings such as nausea and headaches. These were usually followed by arthralgias and pruritus that often presented by 8 hours after treatment. Two participants also developed mild inguinal tenderness at that time. One individual developed a fever at 8 hours post-treatment. In other individuals that developed fevers following treatment, elevated temperatures were present by 12 hours post-treatment. Temperatures often exceeded 39°C and were successfully treated with acetaminophen. Seven individuals developed hematuria and/or proteinuria, 3 at 24 hours post-treatment and the remaining by 48 hours. Hematuria and/or proteinuria resolved by 72 hours in 3 of 7 individuals, and the remainder resolved by 7 days. Abnormal transaminases were confined to AST and followed a similar kinetics of hematuria and/or proteinuria, with all resolving by 7 days after treatment.

Overall, 10 of 12 (83%) individuals in the 3-drug treatment group developed 1 or more objective AEs compared with 6 of 12 (50%, $P = .19$, Fischer exact test) in the 2-drug group. Median number of AEs per person in the 3-drug group was 2 compared

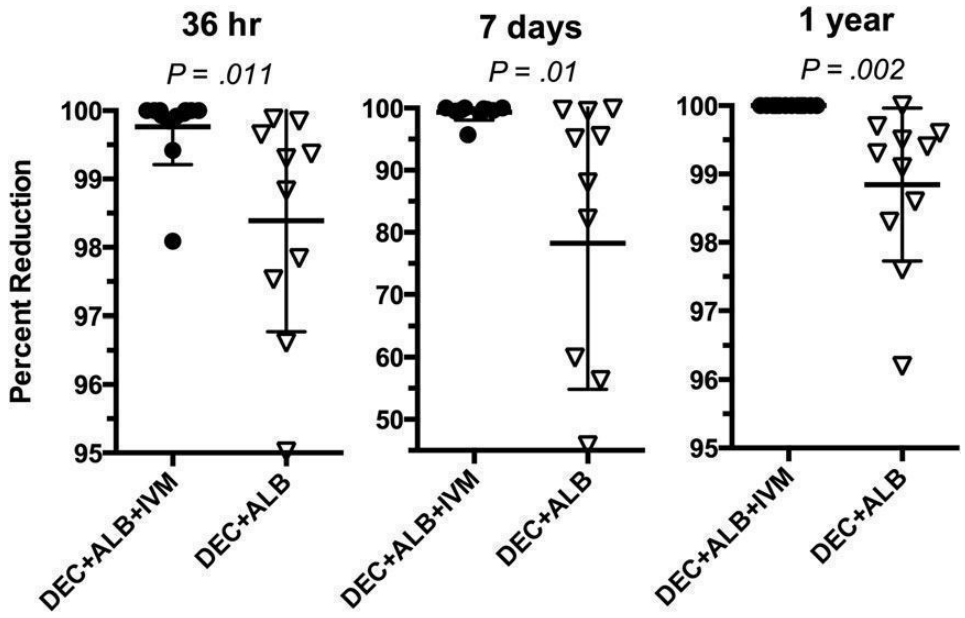

Figure 2. Percent reduction in microfilaria compared to pretreatment levels at 36 hours, 7 days, and 1 year following treatment with diethylcarbamazine (DEC) + albendazole (ALB) + ivermectin (IVM) or DEC + ALB. Microfilaria levels were determined by filtering 1 mL of anticoagulated, nocturnally collected peripheral venous blood through a nucleopore filter. Significance determined by Student *t* test.

Table 2. Microfilarial Levels 2 Years Following a Single Treatment

Treatment	Pre-Treatment	Time Post-Treatment 36 h	7 d	1 y	2 y
Diethylcarbamazine + albendazole	3235[a]	4	9	10	0
	1599	10	8	11	44
	1562	1	627	22	0
	1095	27	49	7	4
	1107	13	1	27	13
	1747	87	947	29	37
Diethylcarbamazine + albendazole + ivermectin	689	0	0	0	0
	677	1	1	0	0
	1034	6	8	0	0
	1476	1	4	0	0
	1857	0	0	0	0
	2509	0	0	0	0

[a] Microfilaria per milliliter of filtered whole blood.

with 0.5 in the 1-drug group ($P = .094$). Hematuria and/or proteinuria also predominantly occurred in the 3-drug treatment group. A scoring system was used based on severity and duration of the hematuria and/or proteinuria (see "Methods" section), with individuals in the 3-drug group having a score of 16 vs 1 in the 2-drug group.

With respect to subjective AEs, the proportion of individuals who developed 1 or more complaints, including headache, nausea, pruritus, abdominal pain, weakness, and arthralgia (Table 2), was similar between the 2 treatment groups (9 of 12 [75%] in the 3-drug group and 7 of 12 [58%] in the 2-drug group). Individuals in the 3-drug group tended to have more subjective AEs (median = 2.5) compared with those in the 2-drug group (median = 1, $P = .091$).

When both subjective and objective AEs were combined, individuals on 3-drug therapy had significantly more AEs (median = 4.5) compared with those receiving 2-drug treatment (median = 2.5, $P = .021$). Microfilaremia levels prior to treatment (or closely related reduction in microfilaria levels at 36 hours) were related to the number of AEs independent of treatment ($P = .09$).

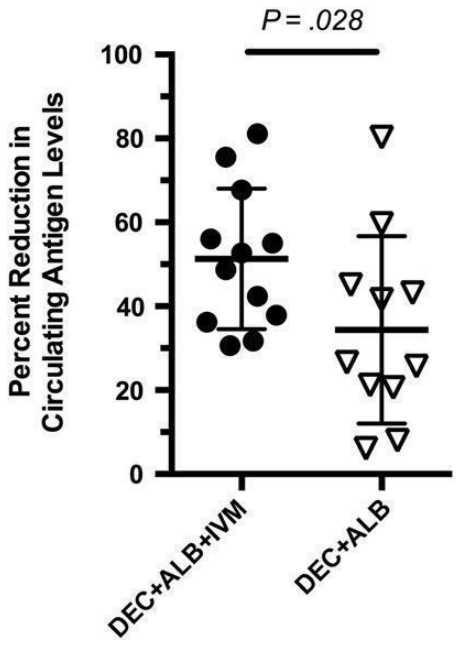

Figure 3. Percent reduction in serum filarial antigen levels 1 year following treatment compared to levels before treatment in participants receiving a single dose of diethylcarbamazine (DEC) + albendazole (ALB) + ivermectin (IVM) or DEC + ALB. Antigen levels were measure using the Og4C3 assay. Significance determined by Student *t* test.

Drug Interactions and Pharmacokinetics

Drug concentration–time curves are shown in Figure 4 and Supplementary Figure 1. The pharmacokinetic parameter estimates for DEC, ALB, ALBSO, and $ALBO_2$ with and without IVM administration are presented in Supplementary Table 1, along with parameter estimates for IVM. No significant drug interactions were observed ($P > .05$ for all treatment group comparisons).

Geometric mean parameter ratios [(with IVM)/(without IVM)] of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ for each analyte (DEC, ALBSO, and $ALBSO_2$) are presented with 90% confidence intervals (CIs) in Supplementary Table 2. DEC C_{max} levels in the 2 treatment groups almost met the commonly used limits for no clinically relevant effect (80%–125%) [17–19]. DEC AUC_{0-t} and $AUC_{0-\infty}$ 90% CIs obtained in this study were only slightly outside of these limits but were completely within the slightly less stringent limits of 70%–143%. Ninety percent CIs for the parameter estimates of ALBSO and $ALBSO_2$ were quite broad, because of the small samples sizes in this study.

DISCUSSION

In this study, we compared the effects of a new triple-drug regimen (IVM + DEC + ALB) with standard DEC + ALB as a

Table 3. Number and Frequency of Adverse Events Experienced by Study Participants After Treatment

Adverse Event	Regimen	
	Diethylcarbamazine + Albendazole (N = 12)	Diethylcarbamazine + Albendazole + Ivermectin (N = 12)
Objective findings		
Fever	2 (17)	6 (50)
Lymphadenitis	5 (42)	3 (25)
Hepatic (alanine transaminase \aspartate transaminase) abnormalities[a]	2 (17)	1 (8
Proteinuria	1 (8)	3 (25)
Hematuria	0 (0)	4 (33)
Transient low blood pressure	1 (8)	1 (8)
Subjective findings		
Headache	3 (25)	6 (50)
Joint pain	5 (42)	7 (58)
Nausea	1 (8)	3 (25)
Cough	0 (0)	5 (42)
Abdominal pain	1 (8)	4 (33)
Itch	3 (25)	5 (42)
Weakness	0 (0)	2 (17)
Dizziness	2 (17)	1 (8)

[a] >1.5 times the upper limit of normal.

single-dose treatment for Bancroftian filariasis in treatment-naive participants with high-intensity *W. bancrofti* infections. Triple therapy rapidly eliminated almost all microfilaria from peripheral blood, and, importantly, all participants treated with this regimen were amicrofilaremic 1 and 2 years following treatment. Microfilaria counts also rapidly declined in participants treated with DEC + ALB but less dramatically than in those treated with triple therapy. Also, the 2-drug therapy failed to clear microfilaria in most participants 12 and 24 months after treatment, which is consistent with results from other treatment trials [7–9, 11]. A greater reduction in circulating antigen levels with the triple-drug regimen suggests a higher percentage of adult female worms were killed compared with use of DEC + ALB. Since all treatment participants in both groups had persistently positive filarial antigen tests 1 and 2 years after treatment, neither treatment killed all adults worms [20]. The absence of microfilaria at 1 and 2 years after treatment suggests the triple-drug therapy had embryostatic and/or embryocidal effects on adult female worms.

Participants treated with the triple-drug regimen experienced more AEs than those who received DEC + ALB when both objective and subjective AEs were combined. All AEs were mild to moderate in severity, started 8 hours following treatment, peaked at between 12 and 48 hours, and resolved 7 days later, except in 1 participant who had right inguinal tenderness at day 7. AEs observed were consistent with the well-documented transient

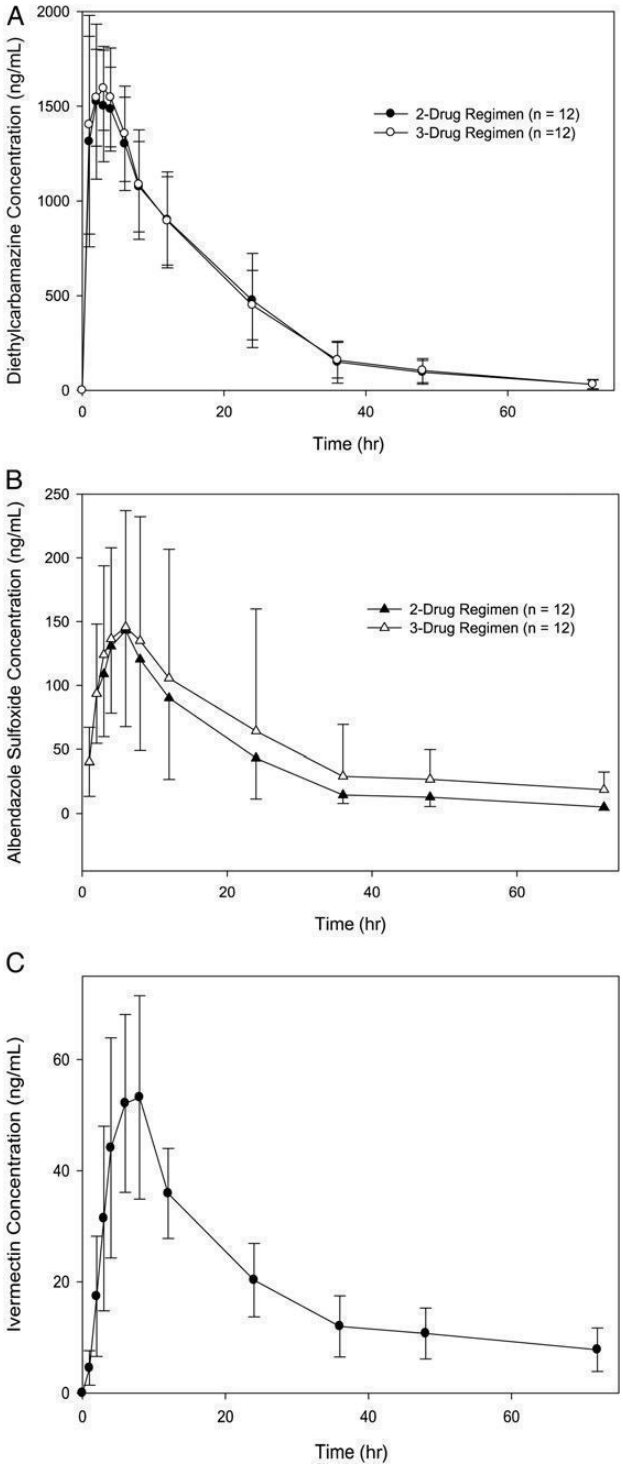

Figure 4. Mean serum plasma levels (± standard deviation) of (*A*) diethylcarbamazine (DEC), (*B*) albendazole (ALB) sulfoxide and (*C*) ivermectin (IVM) at different times following treatment with DEC + ALB + IVM or DEC + ALB.

AEs that have been reported following treatments that kill microfilaria [7–9]. These AEs include fever, headache, pruritus, arthralgia, tender lymph nodes, and development of proteinuria and/or hemoglobinuria. The spectrum of AEs was similar in the

2 treatment arms, except for urinary abnormalities, which were present almost exclusively in the 3-drug treatment group. Development of proteinuria and/or hemoglobinuria in urine ranged from mild to major, as measured by dipstick, and often persisted for several days. All urine abnormalities resolved by 7 days following treatment. No participant had a significant increase in serum creatinine after treatment. Transient urinary abnormalities were similar to those observed in a previous study following treatment with DEC alone [21]. The proteinuria and/or hemoglobinuria may arise from inflammation due to dead microfilaria in the kidney. Alternatively, participants may develop a transient immune complex associated glomerulonephritis, as based on prior studies in animal models of filariasis [22], renal histology [23–26], and presence of filariasis-specific immune complexes in blood and urine [27] of participants with LF.

Pharmacokinetic studies were performed to determine whether IVM affects drug levels or clearance of DEC and ALB. The absorption and disposition profiles of each drug (and/or ALB metabolites) were not significantly different in participants who received IVM. The pharmacokinetics of DEC and IVM were similar to those seen in previous studies [19, 28, 29]. The pharmacokinetics of ALB in this study was also consistent with that seen in prior studies that reported that ALB is poorly absorbed and rapidly eliminated, primarily through metabolism to ALBSO and $ALBSO_2$ [19, 28, 29]. The wide variation in ALBSO levels after ALB is well known and likely due to differences in absorption of the drug between individuals and differences in ALB metabolism between men and women [30]. There was a clear lack of significant interaction between DEC and IVM. Because of high interpatient variability in ALBSO exposure parameters, definitive conclusions cannot be drawn regarding the lack of an effect of IVM on ALBSO exposure. However, the point estimates for the ratios of geometric means are not suggestive of a major influence of IVM on ALBSO exposure, and in a previous analysis, Awadzi et al [28] failed to detect any substantial interaction between these drugs. Thus, it is unlikely that coadministration of IVM has a clinically significant drug interaction with ALB/ALBSO. It has historically been assumed that the minor metabolite $ALBSO_2$ is inactive against filarial parasites because of its relatively lower abundance in human serum compared with ALBSO and because of the known activity of ALB and ALBSO as antagonists of microtubule formation in nematodes [31]. However, a recent study reported that $ALBSO_2$ prevents binary fission in *Wolbachia*, an obligate endosymbiont of *W. bancrofti* [32]. The present analysis is the first to quantify $ALBSO_2$ pharmacokinetics in patients with *W. bancrofti* infection.

This study has shown that single-dose treatment with IVM + DEC + ALB is safe and more effective for clearing *W. bancrofti* microfilaria and reducing filarial antigen levels than standard treatment with DEC + ALB. Additional studies are needed to determine the duration of microfilaria clearance after IVM + DEC + ALB and to further establish the safety of this regimen. A "one and done" regimen could have a transformative impact on the global program to eliminate LF by reducing the number of rounds of MDA required to reach elimination targets. This would be especially useful for countries such as Papua New Guinea where it is extremely difficult to provide repeated rounds of MDA to LF-endemic populations, and it could improve chances for global elimination of LF by the target year of 2020.

Supplementary Data

Supplementary materials are available at http://cid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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